EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	30	hirudin$.ab. and (Gln or glutamine)and mutat$	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 14:58
L2	133	hirudin$.ab. and fragment	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 15:04
L3	14	hirudin$.ab. and digest	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 14:58
L4	66	hirudin$.ab. and fragment.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 15:04
L5	66	hirudin$.ab. and fragment$.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 15:18
L6	31	hirudin$.ab. and trypsin	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 15:18
L7	9	hirudin$.ab. and trypsin.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/11/19 15:18

10560918
File 5:Biosis Previews(R) 1926-2007/Sep W1
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Set	Items	Description
? s hirudin?(2w)peptide?		
	9454	HIRUDIN?
	375450	PEPTIDE?
S1	70	HIRUDIN?(2W)PEPTIDE?

Sept 07

? t s1/7/50-70

1/7/50
DIALOG(R)File 5:Biosis Previews(R)

11165487 BIOSIS NO.: 199293008378
EFFECT OF THE %%%HIRUDIN%%% CARBOXY-TERMINAL %%%PEPTIDE%%% 54-65 ON THE INTERACTION OF THROMBIN WITH PLATELETS
AUTHOR: JANDROT-PERRUS M (Reprint); HUISSE M-G; KRSTENANSKY J L; BEZEAUD A; GUILLIN M-C
AUTHOR ADDRESS: LABORATOIRE DE RECHERCHE SUR L'HEMOSTASE, FACULTE XAVIER BICHAT, 16 RUE HENRI HUCHARD, 75018 PARIS, FRANCE**FRANCE
JOURNAL: Thrombosis and Haemostasis 66 (3): p300-305 1991
ISSN: 0340-6245
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The carboxy-terminal region of hirudin (residues 54-65) has previously been shown to inhibit thrombin clotting activity without binding to the catalytic site of the enzyme. In the present study, the effect of hirudin 54-65 on thrombin interaction with specified platelet proteins has been investigated. Hirudin 54-65 was found to inhibit thrombin-induced platelet aggregation and secretion in a dose-dependent manner. Substitution of either Phe56, Glu57, Ile59, Pro60 or Leu64 showed that these residues were critical for inhibition of thrombin-induced platelet activation whereas sulfation of Tyr63 increased the inhibitory potency of the peptide. Hydrolysis of glycoprotein V, a platelet membrane substrate for thrombin, was only partially inhibited by hirudin 54-65. Although hirudin 54-65 did not decrease the amount of thrombin bound to platelets during cross-linking experiments, it was found to inhibit the specific binding of thrombin to platelet glycoprotein Ib. Since the carboxy-terminal region of hirudin has previously been reported to bind near the trypsin-catalyzed .beta. cleavage site, we have analyzed the consequences of .alpha. to .beta.-thrombin conversion on both thrombin-hirudin 54-65 interaction and thrombin activity toward platelets. The .beta. cleavage induced a decrease in the affinity of thrombin for both glycoprotein Ib and hirudin 54-65. Altogether, our results indicate that thrombin recognition sites for hirudin 54-65 and platelet membrane glycoprotein Ib share common structures located near the .beta. cleavage site at Arg 73 on the thrombin B chain.

1/7/51
DIALOG(R)File 5:Biosis Previews(R)

11158414 BIOSIS NO.: 199293001305
THE AMINO-TERMINAL ACIDIC DOMAIN OF HEPARIN COFACTOR II MEDIATES THE INHIBITION OF ALPHA THROMBIN IN THE PRESENCE OF GLYCOSAMINOGLYCANS
AUTHOR: VAN DEERLIN V M D (Reprint); TOLLEFSEN D M
AUTHOR ADDRESS: DIV HEMATOL ONCOL, BOX 8125, WASHINGTON UNIV MED SCH, 660 S EUCLID AVE, ST LOUIS, MO 63110, USA**USA
JOURNAL: Journal of Biological Chemistry 266 (30): p20223-20231 1991
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Heparin cofactor II (HCII) is a glycoprotein in human plasma that inhibits thrombin and chymotrypsin. Inhibition occurs when the protease attack the reactive site peptide bond in HCII (Leu444-Ser445) and becomes trapped as a covalent 1:1 complex. Dermatan sulfate and heparin increase the rate of inhibition of thrombin, but not of chymotrypsin, > 1000-fold. The N-terminal portion of HCII contains two acidic repeats (Glu56-Asp-Asp-Asp-Tyr-Leu-Asp and Glu69-Asp-Asp-Asp-Tyr-Ile-Asp) that may bind to anion-binding exosite I of thrombin to facilitate covalent complex formation. To examine the importance of the acidic domain, we have constructed a series of 5' deletions in the HCII cDNA and expressed the recombinant HCII (rHCII) in Escherichia coli. Apparent second-order rate constants (k2) for inhibition of .alpha.-thrombin and chymotrypsin by each variant were determined. Deletion of amino acid residues 1-74 had no effect on the rate of inhibition of .alpha.-thrombin or chymotrypsin in the absence of a glycosaminoglycan. Similarly, the rate of inhibition of .alpha.-thrombin in the presence of a glycosaminoglycan was unaffected by deletion of residues 1-52. However, deletion of residues 1-67 (first acidic repeat) or 1-74 (first and second acidic repeats) greatly decreased the rate of inhibition of .alpha.-thrombin in the presence of heparin, dermatan sulfate, or a dermatan sulfate hexasaccharide that comprises the minimum high-affinity binding site for HCII. Deletion of one or both of the acidic repeats increased the apparent affinity of rHCII for heparin-Sepharose, suggesting that the acidic domain may interact with the glycosaminoglycan-binding site of native rHCII. The stimulatory effect of glycosaminoglycans on native rHCII was decreased by a C-terminal %%%hirudin%%% %%%peptide%%% which binds to anion-binding exosite I of .alpha.-thrombin. Furthermore, the ability of native rHCII to inhibit .gamma.-thrombin, which lacks the binding site for hirudin, was stimulated weakly by glycosaminoglycans. These results support a model in which the stimulatory effect of glycosaminoglycans on the inhibition of .alpha.-thrombin is mediated, in part, by the N-terminal acidic domain of HCII.

1/7/52

10877522 BIOSIS NO.: 199192123293
THE REGION OF THE THROMBIN RECEPTOR RESEMBLING HIRUDIN BINDS TO THROMBIN AND ALTERS ENZYME SPECIFICITY
AUTHOR: LIU L-W (Reprint); VU T-K H; ESMON C T; COUGHLIN S R
AUTHOR ADDRESS: HOWARD HUGHES MEDICAL INSTITUTE, OKLAHOMA MEDICAL RESEARCH FOUNDATION, 820 NE 15TH ST, OKLAHOMA CITY, OKLA 73104, USA**USA

JOURNAL: Journal of Biological Chemistry 266 (26): p16977-16980 1991
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A thrombin receptor has recently been cloned and the sequence deduced. The sequence reveals a thrombin cleavage site that accounts for receptor activation. The receptor also has an acidic region with some similarities to the carboxyl-terminal region of the leech thrombin inhibitor, %%%hirudin%%%. Synthetic %%%peptides%%% corresponding to the receptor cleavage site (residues 38-45), the hirudin-like domain (residues 52-69), and the covalently associated domains (residues 38-64) were evaluated for their ability to bind to thrombin. Peptides 38-45 and 38-64 were competitive inhibitors of thrombin's chromogenic substrate activity (Ki = 0.96 mM and 0.6 .mu.M, respectively). Residues 52-69 altered the chromogenic substrate specificity, resulting in accelerated cleavage of some substrates and inhibited cleavage of others. The same peptide binds to thrombin and alters the fluorescence emission intensity of 5-dimethylaminonaphthalene-1-sulfonyl (dansyl)-thrombin in which the dansyl is attached directly to the active site serine (Kd = 32 .+-. 7 .mu.M). Residues 52-69 displace the carboxyl-terminal peptide of hirudin, indicating that they share a common binding site in the anion exosite of thrombin. These data suggest that the thrombin due to the presence of the hirudin-like domain and that this domain alters the specificity of thrombin. This change in specificity may account for the ability of the receptor to severe as an excellent thrombin substrate despite the presence of an Asp residue in the P3 site, which is normally inhibitory to thrombin activity.

10821877 BIOSIS NO.: 199192067648
DECIPHERING THE STRUCTURAL ELEMENTS OF %%%HIRUDIN%%% CARBOXYL-TERMINAL %%%PEPTIDE%%% THAT BIND TO THE FIBRINOGEN RECOGNITION SITE OF ALPHA THROMBIN
AUTHOR: CHANG J-Y (Reprint)
AUTHOR ADDRESS: PHARMACEUTICALS RESEARCH LABORATORY, CIBA-GEIGY LTD, BASEL CH-4002, SWITZ**SWITZERLAND
JOURNAL: Biochemistry 30 (27): p6656-6661 1991
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The C-terminal peptide of a hirudin acts as an anticoagulant by binding specifically to a noncatalytic (fibrinogen recognition) site of thrombin. This binding has been shown to shield five spatially distant lysines of the thrombin B-chain (Lys21, Lys 65, Lys77, Lys106, and Lys 107). It was also demonstrated that modification of the sequence of the %%%hirudin%%% C-terminal %%%peptide%%% invariably diminished its anticoagulant that modification of the sequence of the hirudin h/C-terminal peptide invariably diminished its anticoagulant activity. The major object of this study is to investigate how the decreased

activity of the modified %%%hirudin%%% C-terminal %%%peptide%%% is reflected by the changes of its binding properties to these five lysines of thrombin. A synthetic peptide representing the last 12 C-terminal amino acids of hirudin (Hir54-65) was (1) truncated from both its N-terminal and its C-terminal ends, or (2) substituted with Gly along residues 57-62, or (3) chemically modified to add (sulfation at Tyr63) or abolish (Asp and Glu modification with carbodimide/glycinamide) its negatively charges side chains. The binding characteristics of these peptides to thrombin were investigated by chemical methods, and their corresponding anticoagulant activities were studied. Our results demonstrated the following (1) the anticoagulant activities of %%%hirudin%%% C-terminal %%%peptides%%% were quantitatively related to their abilities to shield the five identified lysines of thrombin. The most potent peptide was sulfated Hir54-65 (S-Hir54-65) with an average binding affinity to the five lysines of 120 nM. A heptapeptide (Hir54-60) also displayed anticoagulant activity and thrombin binding ability at micromolar concentrations. (2) All active %%%hirudin%%% C-terminal %%%peptides%%% regardless of their sizes and potencies were shown to be capable of shielding the five lysines of thrombin. The results are discussed in relation to the recently elucidated X-ray model of the hirudin/thrombin complex. Futhermore, the stability of S-Hir54-65 and its relative anticoagulant potency to the N-terminal core fragment of hirudin were examined.

1/7/54
DIALOG(R)File 5:Biosis Previews(R)

10727731 BIOSIS NO.: 199191110622
POSITIONAL EFFECTS OF SULFATION IN HIRUDIN AND HIRUDIN PA RELATED ANTICOAGULANT PEPTIDES
AUTHOR: PAYNE M H (Reprint); KRSTENANSKY J L; YATES M T; MAO S J T
AUTHOR ADDRESS: SYNTEX RES, 3401 HILLVIEW AVE, PALO ALTO, CALIF 94303, USA **USA
JOURNAL: Journal of Medicinal Chemistry 34 (3): p1184-1187 1991
ISSN: 0022-2623
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: C-Terminal fragment analogues of the leech protein hirudin or the related protein hirudin PA block thrombin's cleavage of fibrinogen. Three series of synthetic peptides were synthesized to study the effects of sulfation in %%%hirudin%%%-derived %%%peptides%%%. Potency of hirudin analogues increased with p-(amino)Phe63, p-(aminosulfonate)Phe63, and p-(sulfate)Tyr63 substitution in place of Tyr63. Sulfation of Tyr56, which in hirudin is normally Phe, resulted in a loss of 1 order of magnitude in potency. The sulfation of Tyr64 of the hirudin PA related analogues resulted in increased potency as for the hirudin analogue. However, in this series the p-(amino)Phe64 and p-(aminosulfonate)Phe64 did not have increased potency. In addition to these positional effects, replacing all the Glu residues with (O-sulfato)Ser yielded an analogue with full antithrombin potency.

1/7/55

DIALOG(R)File 5:Biosis Previews(R)

10716260 BIOSIS NO.: 199191099151
SELECTIVE INHIBITION BY A SYNTHETIC %%%HIRUDIN%%% %%%PEPTIDE%%% OF FIBRIN-DEPENDENT THROMBOSIS IN BABOONS
AUTHOR: CADROY Y (Reprint); MARAGANORE J M; HANSON S R; HARKER L A
AUTHOR ADDRESS: DIVISION HEMATOLOGY/ONCOLOGY, EMORY UNIVERSITY SCHOOL MEDICINE, PO DRAWER AR, ATLANTA, GA 30322, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 88 (4): p1177-1181 1991
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: To determine the importance of the thrombin substrate recognition exosite for fibrinogen binding in the formation of both arterial and venous thrombi, we evaluated the antithrombotic effects of the tyrosine-sulfated dodecapeptide from residues 53-64 of %%%hirudin%%% (H %%%peptide%%%) in a nonhuman primate model. This peptide was studied because it inhibits thrombin cleavages of fibrinogen by simple competition with out blocking enzyme catalytic-site function. When an exteriorized arteriovenous access shunt model was used in baboons (Papio anubis), thrombus formation was induced by placing a thrombogenic device made of (i) a segment of tubing coated covalently with type I collagen, which generated platelet-rich thrombi under arterial flow conditions, and (ii) two subsequent annular regions of flow expansion that produced fibrin-rich thrombi typically associated with venous valves and veins. Thrombus formation was quantified by measurements of 111In-labeled platelet and 125I-labeled fibrinogen deposition in both arterial-flow and venous-flow portions of the device. Continuous infusion of H peptide (0.5, 15, and 75 mg/kg) proximal to the device for 40 min interrupted, in a dose-response fashion, formation of fibrin-rich thrombus in the regions of disturbed flow and generation of fibrinopeptide A. In contrast, H peptide did not inhibit the capacity of platelets to deposit on the collagen surface ($P < 0.2$ at all doses) or to form hemostatic plugs (as assessed by measurements of bleeding time; $P > 0.01$ at all doses). These findings suggest that, by competitive inhibition of fibrinogen binding to thrombin, fibrin-rich venous-type thrombus formation may be selectively prevented. This strategy may be therapeutically attractive for preserving normal platelet function when conventional anticoagulant therapy is contraindicated.

1/7/56
DIALOG(R)File 5:Biosis Previews(R)

10705285 BIOSIS NO.: 199191088176
ROLE OF NEW ANTICOAGULANTS AS ADJUNCTIVE THERAPY DURING THROMBOLYSIS
AUTHOR: EISENBERG P R (Reprint)
AUTHOR ADDRESS: CARDIAC CARE UNIT, CARDIOVASC DIV, BOX 8086, WASHINGTON UNIV SCH MED, 660 SOUTH EUCLID AVE, ST LOUIS, MISSOURI 63110, USA**USA
JOURNAL: American Journal of Cardiology 67 (3): p19A-24A 1991
ISSN: 0002-9149
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Procoagulant activity may persist during coronary thrombolysis and result in either delay in the time to recanalization or recurrent thrombosis. Although heparin and aspirin form the mainstay of current therapy, recurrent thrombosis occurs despite adjunctive heparin therapy during thrombolysis. Newer agents that inhibit thrombin by antithrombin III-independent mechanisms, or that inhibit earlier steps in the coagulation cascade, have been shown to be effective in the experimental preparation of coronary thrombolysis. Because heparin-antithrombin III is a relatively inefficient inhibitor of thrombin bound to fibrin, agents such as %%%hirudin%%% or small %%%peptide%%% inhibitors of the thrombin-active site appear to be more effective inhibitors of . clot-associated thrombin activity. Inhibition of early steps in the coagulation cascade with the inhibitor of tissue factor-factor VIIa complex, or with activated protein C, also appears to be an effective anticoaglutant strategy. In experimental preparations all of these agents have shown superiority in preventing recurrent thrombosis compared with heparin, and in some cases they appear to accelerate the rate of clot lysis.

1/7/57
DIALOG(R)File 5:Biosis Previews(R)

10664029 BIOSIS NO.: 199191046920
EQUILIBRIUM BINDING OF THROMBIN TO RECOMBINANT HUMAN THROMBOMODULIN EFFECT OF HIRUDIN FIBRINOGEN FACTOR VA AND PEPTIDE ANALOGUES
AUTHOR: TSIANG M (Reprint); LENTZ S R; DITTMAN W A; WEN D; SCARPATI E M; SADLER J E
AUTHOR ADDRESS: HOWARD HUGHES MED INST, WASH UNIV SCH MED, 660 S EUCLID AVE, BOX 8045, ST LOUIS, MO 63110, USA**USA
JOURNAL: Biochemistry 29 (47): p10602-10612 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Thrombomodulin is an endothelial cell surface receptor for thrombin that acts as a physiological anticoagulant. The properties of recombinant human thrombomodulin were studied in COS-7, CHO, CV-1, and K562 cell lines. Thrombomodulin was expressed on the cell surface as shown by the acquisition of thrombin-dependent protein C activation. Like native thrombomodulin, recombinant thrombomodulin contained N-linked oligosaccharides, had Mr .times. 100 000, and was inhibited or immunoprecipitated by anti-thrombomodulin antibodies. Binding studies demonstrated that nonrecombinant thrombomodulin expressed by A549 carcinoma cells and recombinant thrombomodulin expressed by CV-1 and K562 cells had similar Kd's for thrombin of 1.3 nM, 3.3 nM, and 4.7 nM, respectively. The Kd for DIP-thrombin binding to recombinant thrombomodulin on CV-1 (18A) cells was identical with that of thrombin. Increasing concentrations of hirudin or fibrinogen progressively inhibited the binding of 125I-DIP-thrombin, while factor Va did not inhibit binding. Three synthetic peptides were tested for ability to inhibit DIP-thrombin binding. Both the %%%hirudin%%% %%%peptide%%%

Hir53-64 and the thrombomodulin fifth-EGF-domain peptide Tm426-444 displaced DIP-thrombin from thrombomodulin, but the factor V peptide FacV30-43 which is similar in composition and charge to Hir53-64 showed no binding inhibition. The data exclude the significant formation of a ternary complex consisting of thrombin, thrombomodulin, and hirudin. These studies are consistent with a model in which thrombomodulin, hirudin, and fibrinogen compete for binding to DIP-thrombin at the same site.

1/7/58
DIALOG(R)File 5:Biosis Previews(R)

10537613 BIOSIS NO.: 199141050239
THROMBIN INHIBITION BY SYNTHETIC %%%HIRUDIN%%% %%%PEPTIDES%%%
BOOK TITLE: LIU, C. Y. AND S. CHIEN (ED.). ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, VOL. 281. FIBRINOGEN, THROMBOSIS, COAGULATION, AND FIBRINOLYSIS; INTERNATIONAL SCIENTIFIC SYMPOSIUM, TAIPEI, TAIWAN, AUGUST 30-SEPTEMBER 1, 1989. X+450P. PLENUM PRESS: NEW YORK, NEW YORK, USA; LONDON, ENGLAND, UK. ILLUS
AUTHOR: MARAGANORE J M (Reprint); FENTON J W II
AUTHOR ADDRESS: BIOGEN INC, 14 CAMBRIDGE CENT, CAMBRIDGE, MASS 02142, USA** USA
SERIES TITLE: Advances in Experimental Medicine and Biology p177-184 1990
ISSN: 0065-2598 ISBN: 0-306-43726-0
DOCUMENT TYPE: Book; Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

1/7/59
DIALOG(R)File 5:Biosis Previews(R)

10373994 BIOSIS NO.: 199140016885
%%%HIRUDIN%%% CARBOXYL-TERMINAL %%%PEPTIDE%%% H54-65 EFFECT ON THROMBIN INTERACTION WITH FIBRINOGEN AND PLATELETS
AUTHOR: JANDROT-PERRUS M (Reprint); HUISSE M-G; BEZEAUD A; GUILLON M-C
AUTHOR ADDRESS: FACULTE XAVIER BICHAT, PARIS, FRANCE**FRANCE
JOURNAL: Circulation 82 (4 SUPPL. 3): pIII659 1990
CONFERENCE/MEETING: 63RD SCIENTIFIC SESSIONS OF THE AMERICAN HEART ASSOCIATION, DALLAS, TEXAS, USA, NOVEMBER 12-15, 1990. CIRCULATION.
ISSN: 0009-7322
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

1/7/60
DIALOG(R)File 5:Biosis Previews(R)

10318248 BIOSIS NO.: 199090102727
DESIGN AND CHARACTERIZATION OF HIRULOGS A NOVEL CLASS OF BIVALENT PEPTIDE INHIBITORS OF THROMBIN
AUTHOR: MARAGANORE J M (Reprint); BOURDON P; JABLONSKI J; RAMACHANDRAN K L;

FENTON J W
AUTHOR ADDRESS: BIOGEN, INC, 14 CAMBRIDGE CENTER, CAMBRIDGE, MASS 02142, USA**USA
JOURNAL: Biochemistry 29 (30): p7095-7101 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A novel class of synthetic peptides has been designed that inhibit the thrombin catalytic site and exhibit specificity for the anion-binding exosite (ABE) of .alpha.-thrombin. These peptides, called "hirulogs", consist of (i) an active-site specificity sequence with a restricted Arg-Por scissile bond, (ii) a polymer linker of glycyl residues from 6 to 18 .ANG. in length, and (iii) an ABE recognition sequence such as that in the hirudin C-terminus. Hirulog-1 [(D-Phe)-Pro-Arg-Por-(Gly)4-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Tyr-Leu] inhibits the thrombin-catalyzed hydrolysis of a tripeptide p-nitroanilide substrate with Ki = 2.3 nM. In contrast, the synthetic C-terminal %%%hirudin%%% %%%peptide%%% S-Hir53-64, which binds to the thrombin ABE, blocked the fribinogen clotting activity of the enzyme with Ki = 144 nM but failed to inhibit the hydrolysis of p-nitroanilide substrates at concentrations as high as 1 mM. In addition, the pentapeptide (D-Phe)-Pro-Arg-Pro-Gly, which comprises the catalytic-site inhibitor moiety of hirulog-1, was determined to have a Ki for thrombin inhibition >2 .mu.M. Hirulog-1, but not S-Hir53-64, was found to inhibit the incorporation of [14C]diisopropyl fluorophosphate in thrombin. Hirulog-1 appears specific for thrombin as it lacks inhibitory activities toward human factor Xa, human plasmin, and bovine trypsin at inhibitor:enzyme concentrations 3 orders of magnitude higher than those required to inhibit thrombin. The optimal inhibitory activity of hirulog-1 depends upon all three components of its structure. Hirulog-1 inhibited human .gamma.-thrombin and bovine thrombin with Ki values increased 500- and 20-fold, respectively, compared to Ki for human .alpha.-thrombin. Also, hirulog-1 inhibition of .alpha.-thrombin was reversed in the presence of saturating concentrations of S-Hir53-64. Studies on the optimal length of the oligoglycyl spacer, which forms a molecular "bridge" linking active-site and ABE recognition sequences, showed that at least three to four glycines were necessary for optimal inhibitory activity. Comparison of anticoagulant activities of hirulog-1, hirudin, and S-Hir53-64 showed that the synthetic hirulog-1 is 2-fold more potent than hirudin and 100-fold more active than S-Hir53-64 in increasing the activated partial thromboplastin time of normal human plasma. Thus, fashioned from studies on hirudin and its fragments, synthetic peptides that bind to both the ABE and catalytic site of thrombin are potent reversible inhibitors of thrombin activities.

1/7/61
DIALOG(R)File 5:Biosis Previews(R)

10298587 BIOSIS NO.: 199090083066
AFFINITY LABELING OF LYSINE-149 IN THE ANION-BINDING EXOSITE OF HUMAN ALPHA THROMBIN WITH AN N-ALPHA DINITROFLUOROBENZYLHIRUDIN CARBOXYL TERMINAL PEPTIDE
AUTHOR: BOURDON P (Reprint); FENTON J W II; MARAGANORE J M

AUTHOR ADDRESS: BIOGEN INC, 14 CAMBRIDGE CENT, CAMBRIDGE, MASS 02142, USA**
USA
JOURNAL: Biochemistry 29 (27): p6379-6384 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In order to define structural regions in thrombin that interact with hirudin, the N.alpha.-dinitrofluorobenzyl analogue of an undecapeptide was synthesized corresponding to residues 54-64 of hirudin [GDFEEIPEEY(O35SO3)L (DNFB-[35S]Hir54-64)]. DNFB-[35S]Hir54-64 was reacted at a 10-fold molar excess with human .alpha.-thrombin in phosphate-buffered saline at pH 7.4 and 23.degree.C for 18 h. Autoradiographs of the product in reducing SDS-polyacrylamide gels revealed a single 35S-labeled band of Mr .apprx.32 500. The labeled product was coincident with a band on Coomassie Blue stained gels migrating slightly above an unlabeled thrombin band at Mr .apprx.31 000. Incorporation of the 35S affinity reagent peptide was found markedly reduced when reaction with thrombin was performed in the presence of 5- and 20-fold molar excesses of unlabeled %%%hirudin%%% %%%peptide%%%, showing that a specific site was involved in complex formation. The human .alpha.-thrombin-DNFB-Hir54-64 complex was reduced, S-carboxymethylated, and treated with pepsin. Peptic fragments were separated by reverse-phase HPLC revealing two major peaks containing absorbance at 310 nm. Automated Edman degradation of the peptide fragments allowed identification of Lys-149 of human thrombin as the major site of DNFB-Hir54-64 derivatization. These data suggest that the anionic C-terminal tail of hirudin interacts with an anion-binding exosite in human thrombin removed 18-20 .ANG. from the catalytic apparatus.

10269716 BIOSIS NO.: 199090054195
%%%HIRUDIN%%%-BASED %%%PEPTIDES%%% BLOCK THE INFLAMMATORY EFFECTS OF THROMBIN ON ENDOTHELIAL CELLS
AUTHOR: PRESCOTT S M (Reprint); SEEGER A R; ZIMMERMAN G A; MCINTYRE T M; MARAGANORE J M
AUTHOR ADDRESS: CVRTI, BUILD 100, UNIV UTAH, SALT LAKE CITY, UTAH, 84112
JOURNAL: Journal of Biological Chemistry 265 (17): p9614-9616 1990
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Thrombin is a serine protease that plays an essential role in blood coagulation and also induces various responses in endothelial cells. The actions of thrombin on the conversion of fibrinogen to fibrin are inhibited by peptides based on the amino acid sequence of hirudin, a natural anticoagulant from leeches. We show in these studies that the peptides Hir45-64 and sulfated Hir53-64 block the effects of thrombin on endothelial cells. These peptides inhibited, in a concentration-dependent manner, the synthesis of prostaglandin I2 and platelet-activating factor, and the acquisition of an adhesive surface for leukocytes that occur in

response to thrombin. These actions of the peptides occurred even though the catalytic site of thrombin was not blocked.

1/7/63
DIALOG(R)File 5:Biosis Previews(R)

10246971 BIOSIS NO.: 199090031450
THROMBIN-BOUND CONFORMATION OF THE CARBOXYL-TERMINAL FRAGMENTS OF HIRUDIN DETERMINED BY TRANSFERRED NUCLEAR OVERHAUSER EFFECTS
AUTHOR: NI F (Reprint); KONISHI Y; SCHERAGA H A
AUTHOR ADDRESS: SECT PROTEIN ENG, BIOTECHNOL RES INST, NATL RES COUNCIL CANADA, MONTREAL, QUEBEC, CANADA H4P 2R2**CANADA
JOURNAL: Biochemistry 29 (18): p4479-4489 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The interaction of the C-terminal fragments (residues 52-65 and 55-65) of the thrombin-specific inhibitor hirudin with bovine thrombin was studied by use of one- and two-dimensional NMR techniques in aqueous solution. Thrombin induces specific line broadening of the proton resonances of residues Asp(55) to Gln(65) of the synthetic hirudin fragments H-Asn
-Asp-gly-Asp(55)-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr(63)-Leu-Gln-COOH and acetyl-Asp(55)-PHe-GLu-GLu-Ile-Pro-gLu-Glu-Tyr(63)-Leu-gLn-COOH. This demonstrates that residues 55-65 are the predominant binding site of hirudin fragments with thrombin. Hirudin fragments take on a well-defined structure when bound to thrombin as indicated by several long-range transferred NOEs between the backbone and side-chain protons of the peptides, but they are not structured when free in solution. Particularly, transferred NOEs exist between the .alpha.CH proton of Glu(61) and the NH proton of Leu(64)[d.alpha.N(i,ti+3)], between the .alpha.CH proton of Glu(61) and the .beta.CH2 protons of LEu(64) [d.alpha..beta.(i,i+3)], and between the .alpha.CH proton of GLu(62) and the .GAMMA.CH2 protons of GLn(65) [D.alpha..gamma.(I,i+3)]. These NOEs are characteristic of an .alpha.-helical structure involving residues GLu(61) to Gln(65). There are also NOEs between the side-chain protons of residues Phe(56), Ile(599), PRo(60), Tyr(63) and Leu(64). Distance geometry calculations suggest that in the structure of the thrmbin-bound %%%hirudin%%% %%%peptides%%% all the charged residues lie on the opposite side of a hydrophobic cluster formed by the nonpolar side chains of residues Phe(56), Ile(59), Pro(60), Tyr(63), and Leu(64).

1/7/64
DIALOG(R)File 5:Biosis Previews(R)

10200070 BIOSIS NO.: 199089117961
FUNCTIONAL SITES OF GLIA-DERIVED NEXIN GDN IMPORTANCE OF THE SITE REACTING WITH THE PROTEASE
AUTHOR: NICK H (Reprint); HOFSTEENGE J; SHAW E; ROVELLI G; MONARD D
AUTHOR ADDRESS: CIBA GEIGY LTD, K-125 113, 4002 BASEL, SWITZERLAND**SWITZERLAND

JOURNAL: Biochemistry 29 (9): p2417-2421 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Glia-derived nexin (GDN) is a 43-kDa serine protease inhibitor with neurite promoting activity in mouse neuroblastoma cells (Guenther et al., 1985). In chick sympathetic neurons, GDN but not %%%hirudin%%% and synthetic %%%peptide%%% inhibitors promoted neurite outgrowth (Zurn et al., 1988). Thus, it was considered that the protease inhibitory activity cannot account for the total biological activity of GDN. We show here that synthetic peptide inhibitors with thrombin specificity mimic GDN at similar concentrations in neuroblastoma cells. Limited proteolysis of GDN with elastase causes a cleavage between sites P1 and P2, corresponding to residues Ala-344-Arg-345 of the molecule. The resulting fragments still copurify on heparin-Sepharose, but the protease inhibitor activity of GDN and the GDN neurite promoting activity are lost. The results confirm the necessity of an intact reactive site for the biological activity of GDN.

10194258 BIOSIS NO.: 199089112149
PREPARATION OF MONOCLONAL ANTIBODIES TO %%%HIRUDIN%%% AND %%%HIRUDIN%%% %%%PEPTIDES%%% A METHOD FOR STUDYING THE HIRUDIN THROMBIN INTERACTION
AUTHOR: SCHLAEPPI J-M (Reprint); VEKEMANS S; RINK H; CHANG J-Y
AUTHOR ADDRESS: R-1056319, CIBA-GEIGY AG, CH-4002 BASEL, SWITZ**SWITZERLAND
JOURNAL: European Journal of Biochemistry 188 (2): p463-470 1990
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A panel of four monoclonal antibodies was obtained against hirudin, a potent and specific inhibitor of thrombin, by immunizing three groups of mice with protein conjugates made of recombinant desulfatohirudin (group I) or two synthetic peptides representing the C-terminal sequences 40-65 (group II) and 52-65 (group III) of hirudin. Only the monoclonal antibody 4049-83-12, obtained from the group I of mice, showed high affinity for hirudin (Kd of 0.6 nM) and in vitro neutralizing properties. The anti-peptide monoclonal antibodies bound hirudin with lower affinity (Kd of 1.5-7 nM) and showed lower neutralizing capacities. An epitope analysis performed by competitive ELISA using various hirudin analogues and by limited proteolysis of the hirudin-antibody complex revealed that the binding domains of all the anti-pepetide antibodies were located close to the C-terminus of hirudin, since the bond between Glu-61 and Glu-62 was not cleaved by the V8 staphylococcal protease in the presence of these antibodies. The epitope of the antibody 4049-83-12 was strictly conformation-dependent, it recognized neither S-carboxymethylated %%%hirudin%%% nor any %%%peptides%%% of hirudin. The cleavage of the bond between Glu-43 and Gly-44 by V8 protease, as well as the cleavage of the bond betwenn Lys-47 and Pro-48 by lysyl endopeptidase, was prevented by the binding of the antibody 4049-83-12 to hirudin. The possibility that this epitope

overlapped with a region of hirudin involved in the binding to thrombin is discussed.

1/7/66
DIALOG(R)File 5:Biosis Previews(R)

10156580 BIOSIS NO.: 199089074471
INHIBITION OF COAGULATION AND THROMBIN-INDUCED PLATELET ACTIVITIES BY A SYNTHETIC DODECAPEPTIDE MODELED ON THE CARBOXYL-TERMINUS OF HIRUDIN
AUTHOR: JAKUBOWSKI J A (Reprint); MARAGANORE J M
AUTHOR ADDRESS: DEP CARDIOVASCULAR PHARMACOLOGY, LILLY RES LAB, MC 304, LILLY CORPORATE CENTER, INDIANAPOLIS, INDIANA 46285, USA**USA
JOURNAL: Blood 75 (2): p399-406 1990
ISSN: 0006-4971
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A synthetic, tyrosine-sulfated, dodecapeptide (BG8865) modeled on residues 53-64 of hirudin was found to elevate the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of human plasma in a dose-dependent manner. The most sensitive assay was the TT, which was prolonged 2 and 3 times control values at 2.2 and 4.1 .mu.g/mL %%%hirudin%%% %%%peptide%%%, respectively. The sulfated dodecapeptide exhibited no dependency on antithrombin III as monitored by the APTT in the presence of sheep anti-human antithrombin III antibodies, and its activity was not neutralized by platelet releasates or platelet factor 4. In studies of thrombin-induced platelet activation, the %%%hirudin%%% %%%peptide%%% was found to block aggregation, serotonin release and thromboxane A2 generation. At thrombin concentrations of 0.25 U/mL, the IC50 (concentration resulting in 50% inhibition) for inhibition of platelet aggregation was 0.72 .mu.g/mL peptide. Inhibition of TXA2 generation and serotonin release correlated closely with inhibition of aggregation. Using platelets from patients with clinically documented heparin-induced thrombocytopenia anticoagulant doses of heparin were found to induce platelet aggregation and thromboxane A2 generation. In sharp contrast, anticoagulant-equivalent doses of %%%hirudin%%% %%%peptide%%% had no effect on patient platelets, as evidenced by a lack of platelet aggregation and thromboxane A2 generation. These data provide compelling in vitro evidence that the %%%hirudin%%% %%%peptide%%% has several potential advantages over heparin, namely effective inhibition of thrombin-induced platelet activities, co-factor independence, insensitivity to endogenous heparin-neutralizing factors, and an apparent lack of direct or immune-mediated platelet stimulating properties.

1/7/67
DIALOG(R)File 5:Biosis Previews(R)

10073537 BIOSIS NO.: 199039126926
INHIBITION OF THROMBIN BY SYNTHETIC %%%HIRUDIN%%% %%%PEPTIDES%%%
AUTHOR: BINNIE G G (Reprint); ERICKSON B W; HERMANS J
AUTHOR ADDRESS: DEP OF PATHOL, 607 BRINKHOUS-BULLITT BUILDING, UNIV OF NORTH CAROLINA, CHAPEL HILL, NC 27599, USA**USA

JOURNAL: Febs Letters 270 (1-2): p85-89 1990
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: ENGLISH

1/7/68
DIALOG(R)File 5:Biosis Previews(R)

10062290 BIOSIS NO.: 199039115679
THE CARBOXYL-TERMINAL BINDING DOMAIN OF HIRULLIN P18 ANTITHROMBIN ACTIVITY AND COMPARISON TO %%%HIRUDIN%%% %%%PEPTIDES%%%
AUTHOR: KRSTENANSKY J L (Reprint); OWEN T J; YATES M T; MAO S J T
AUTHOR ADDRESS: MERRELL DOW RES INST, 2110 E GALBRAITH ROAD, CINCINNATI, OHIO 45215, USA**USA
JOURNAL: Febs Letters 269 (2): p425-429 1990
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: ENGLISH

1/7/69
DIALOG(R)File 5:Biosis Previews(R)

09777332 BIOSIS NO.: 198988092447
ANTITHROMBIN ACTIVITY OF A PEPTIDE CORRESPONDING TO RESIDUES 54-75 OF HEPARIN COFACTOR II
AUTHOR: HORTIN G L (Reprint); TOLLEFSEN D M; BENUTTO B M
AUTHOR ADDRESS: EDWARD MALLINCKRODT DEP PEDIATR, WASHINGTON UNIV SCH MED, ST LOUIS, MO 63110, USA**USA
JOURNAL: Journal of Biological Chemistry 264 (24): p13979-13982 1989
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Heparin cofactor II (HCII) is a highly specific serine proteinase inhibitor, which complexes covalently with thrombin in a reaction catalyzed by heparin and other polyanions. The molecular basis for the thrombin specificity may be explained by the identification here of a segment of HCII including residues 54-75 that binds to thrombin. A synthetic peptide, HCII(54-75), based on this segment of HCII, Gly-Glu-Glu-Asp-Asp-Asp-Tyr-Leu-Asp-Leu-Glu-Lys-Ile-Phe-Ala-Glu-Asp-Asp-Asp-Tyr-Ile-Asp inhibited thrombin's cleavage of fibrinogen. Clotting activity of thrombin was inhibited 50% at a concentration of 28 .mu.M. Polyacrylamide gel electrophoresis showed that HCII(54-75) inhibited thrombin's cleavage of both the A.alpha. and B.beta. polypeptides in fibrinogen. However, the peptide did not block thrombin's active site, as hydrolysis of chromogenic substrates was not inhibited. HCII(54-75) probably binds to the same site on thrombin as do carboxyl-terminal residues of hirudins, thrombin inhibitors of leeches. HCII(54-75) inhibited binding of thrombin to a synthetic peptide corresponding to residues 54-66 of hirudin PA, but the %%%hirudin%%% %%%peptide%%% was

about 30-fold more potent in binding and clotting assays. Both synthetic peptides, as a result of their polyanionic character, might be expected to stimulate the reaction of HCII with thrombin. However, the %%%hirudin%%%-related %%%peptide%%% inhibited this reaction, suggesting that it blocked a site on thrombin required for interaction with HCII. HCII(54-75) had a net stimulatory effect on the thrombin-HCII reaction as a consequence of its lower affinity for thrombin and greater negative charge relative to the %%%hirudin%%%-related %%%peptide%%%. These studies suggest that residues 54-75 of HCII interact with a noncatalytic binding site on thrombin and that this interaction contributes to efficient inhibition of thrombin by HCII.

09726517 BIOSIS NO.: 198988041632
ANTICOAGULANT ACTIVITY OF SYNTHETIC %%%HIRUDIN%%%. %%%PEPTIDES%%%
AUTHOR: MARAGANORE J M (Reprint); CHAO B; JOSEPH M L; JABLONSKI J; RAMACHANDRAN K L
AUTHOR ADDRESS: DEP PROTEIN CHEM, BIOGEN INC, CAMBRIDGE, MASS 02142, USA** USA
JOURNAL: Journal of Biological Chemistry 264 (15): p8692-8698 1989
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Synthetic peptides based on the COOH-terminal 21 residues of hirudin were prepared in order to 1) evaluate the role of this segment in hirudin action toward thrombin, 2) define the shortest peptide derivative with anticoagulant activity, and 3) investigate the role of tyrosine sulfation in the peptides' inhibitory activities. A hirudin derivative of 20 amino acids, Hir45-64 (derived from residues 45-64 of the hirudin polypeptide), was found to effect a dose-dependent increase in the activated partial thromboplastin time (APTT) of normal human plasma but to have no measurable inhibitory activity toward thrombin cleavage of a tripeptidyl p-nitroanilide substrate. Anticoagulant activity in hirudin derivatives was comparable in peptides of 20, 16, and 12 residues truncated from the NH2 terminus. Additional truncated peptides prepared by synthesis and carboxypeptidase treatment reveal that the minimal sequence of a %%%hirudin%%% %%%peptide%%% fragment with maximal anticoagulant activity is contained within the sequence: NH2-Asn-Glyn-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-COOH. The 12-residue derivative thus identified was reacted with dicyclohexylcarbodiimide in the presence of sulfuric acid to yield a Tyr-sulfated peptide, S-Hir53-64. By comparison to unsulfated peptide, S-Hir53-64. By comparison to unsulfated peptide, S-Hir53-64 was found to contain a specific inhibitory activity enhanced by one order of magnitude toward increase in APTT and to effect a dose-dependent increase in thrombin time of normal human plasma to yield a 4-fold increase in thrombin time with 2.5 .mu.g/ml peptide using 0.8 units/ml .alpha.-thrombin. Comparison of S-Hir53-64 to hirudin in thrombin time and APTT assays reveals a 50-fold differences in molar specific activities toward inhibition of thrombin. Comparison of antithrombin activities of S-Hir53-64 using a variety of animal thrombins demonstrates greatest inhibitory activity toward murine,

rat, and human enzymes and a 10-fold reduced activity toward bovine thrombin.

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? ds

Set     Items   Description
S1         70   HIRUDIN?(2W)PEPTIDE?
?
PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES
? s (Val()Thr()Gly) and(hirudin?(2w)peptide?)
           15853  VAL
           15212  THR
           24858  GLY
              28  VAL(W)THR(W)GLY
            9454  HIRUDIN?
          375450  PEPTIDE?
              70  HIRUDIN?(2W)PEPTIDE?
      S2       0  (VAL()THR()GLY) AND(HIRUDIN?(2W)PEPTIDE?)
? s (Val()Thr()Gly)
           15853  VAL
           15212  THR
           24858  GLY
      S3      28  (VAL()THR()GLY)
? t s3/7/1-28

 3/7/1
DIALOG(R)File   5:Biosis Previews(R)
(c) 2007 The Thomson Corporation. All rts. reserv.

0019433566   BIOSIS NO.: 200700093307
Conserved motif of hepatitis C virus E2/NS1 region
AUTHOR: Anonymous; Weiner Amy J; Houghton Michael
AUTHOR ADDRESS: Benicia, CA USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office
Patents NOV 14 2006 2006
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Immunogenic compositions comprising an immunogenic polypeptide
  and a pharmaceutically acceptable vehicle are described. The immunogenic
  polypeptide comprises the amino acid sequence Xaa-Thr-Xaa-%%%Val%%%-
  %%%Thr%%%-%%%Gly%%%
  -Gly-Xaa-Ala-Ala-Arg-Thr-Thr-Xaa-Gly-Xaa-Xaa-Ser-Leu-Phe-Xaa-Xaa-Gly-Xaa-
  Ser-Gln-Xaa-Ile-Gln-Leu-Ile (SEQ ID NO:8). The immunogenic polypeptide
  can be coupled to a pharmaceutically acceptable carrier, such as a
  diphtheria toxoid.


 3/7/2
DIALOG(R)File   5:Biosis Previews(R)
(c) 2007 The Thomson Corporation. All rts. reserv.

19373079   BIOSIS NO.: 200700032820
Process for reducing an alpha-keto ester
AUTHOR: Anonymous; Moore Jeffrey C; Sturr Michael G; McLaughlin Kathleen;
  Kim Jaehon
```

AUTHOR ADDRESS: Westfield, NJ USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents SEP 19 2006 2006
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A process for preparing (R)-hydroxy ester (II) from alpha-keto ester I comprising adding the alpha-keto ester I to a mixture comprising the ketoreductase enzyme and non-ketoreductase enzyme components, wherein the ketoreductase enzyme has a molecular weight between 36000 and 38000, and wherein the ketoreductase enzyme has an N-terminal amino acid sequence selected from the group of sequences consisting of (SEQ. ID NO. 1) Ala-Ile-Pro-Asp-Asn-Ala-Val-Leu-Glu-Gly-Ser-Leu- Val-Lys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Asn-Gly (SEQ. ID NO. 4) Met-Ala-Ile-Pro-Asp-Asn-Ala-Val-Leu-Glu-Gly-Ser- Leu-Val-Lys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Asn-Gly, and (SEQ. ID NO. 2) Met-Ala-Lys-Ile-Asp-Asn-Ala-Val-Leu-Pro-Glu-Gly- Ser-Leu-Val-Leu-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Asn-Gly.

3/7/3
DIALOG(R)File 5:Biosis Previews(R)

19358722 BIOSIS NO.: 200700018463
Conserved motif of hepatitis C virus E2/NS1 region
AUTHOR: Anonymous; Weiner Amy J; Houghton Michael
AUTHOR ADDRESS: Benicia, CA USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents AUG 29 2006 2006
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Immunogenic polypeptides comprising hepatitis C virus (HCV) immunogens are described. The HCV immunogen comprises the amino acid sequence Xaa-Thr-Xaa-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Gly-Xaa-Ala-Ala-Arg-Thr-Thr-Xaa-Gly-Xaa-Xaa-Ser-Leu-P he-Xaa-Xaa-Gly-Xaa-Ser-Gln-Xaa-Ile-Gln-Leu-Ile (SEQ ID NO:8). The immunogenic polypeptide can be coupled to a bacterial toxoid, such as a diphtheria toxoid.

3/7/4
DIALOG(R)File 5:Biosis Previews(R)

15063622 BIOSIS NO.: 199900323282
Structural recognition of an ICAM-1 peptide by its receptor on the surface of T cells: Conformational studies of cyclo (1, 12)-Pen-Pro-Arg-Gly-Gly-Ser-Val-Leu-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Cys-OH
AUTHOR: Gursoy R N; Jois DSS; Siahaan T J (Reprint)
AUTHOR ADDRESS: Department of Pharmaceutical Chemistry Simons Research Laboratories, The University of Kansas, Lawrence, KS, 66047, USA**USA

JOURNAL: Journal of Peptide Research 53 (4): p422-431 April, 1999 1999
MEDIUM: print
ISSN: 1397-002X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The purpose of this study is to elucidate the solution conformation of cyclic peptide 1 (cIBR), cyclo (1, 12)-Pen 1-Pro2-Arg3-Gly4-Gly5-Ser6-Val7-Leu8-Val9-Thr10-Gly11-Cys12-OH, using NMR, circular dichroism (CD) and molecular dynamics (MD) simulation experiments. cIBR peptide (1), which is derived from the sequence of intercellular adhesion molecule-1 (ICAM-1, CD54), inhibits homotypic T-cell adhesion in vitro. The peptide hinders T-cell adhesion by inhibiting the leukocyte function-associated antigen-1 (LFA-1, CD11a/CD18) interaction with ICAM-1. Furthermore, Molt-3 T cells bind and internalize this peptide via cell surface receptors such as LFA-1. Peptide internalization by the LFA-1 receptor is one possible mechanism of inhibition of T-cell adhesion. The recognition of the peptide by LFA-1 is due to its sequence and conformation; therefore, this study can provide a better understanding for the conformational requirement of peptide-receptor interactions. The solution structure of1 was determined using NMR, CD and MD simulation in aqueous solution. NMR showed a major and a minor conformer due to the presence of cis/trans isomerization at the X-Pro peptide bond. Because the contribution of the minor conformer is very small, this work is focused only on the major conformer. In solution, the major conformer shows a trans-configuration at the Pen1-Pro2 peptide bond as determined by HMQC NMR. The major conformer shows possible beta-turns at Pro2-Arg3-Gly4-Gly5, Gly5-Ser6-Val7-Leu8, and Val9-Thr10-Gly11-Cys12. The first beta-turn is supported by the ROE connectivities between the NH of Gly4 and the NH of Gly5. The connectivities between the NH of Ser6 and the NH of Val7, followed by the interaction between the amide protons of Val7 and Leu8, support the presence of the second beta-turn. Furthermore, the presence of a beta-turn at Val9-Thr10-Gly11-Cys12 is supported by the NH-NH connectivities between Thr10 and Gly11 and between Gly11 and Cys12. The propensity to form a type I beta-turn structure is also supported by CD spectral analysis. The cIBR peptide (1) shows structural similarity at residues Pro2 to Val7 with the same sequence in the X-ray structure of D1-domain of ICAM-1. The conformation of Pro2 to Val7 in this peptide may be important for its binding selectivity to the LFA-1 receptor.

3/7/5

14826848 BIOSIS NO.: 199900086508
Properties of a cold-active protease from psychotrophic Flavobacterium balustinum P104
AUTHOR: Morita Y; Hasan Q; Sakaguchi T; Murakami Y; Yokoyama K; Tamiya E (Reprint)
AUTHOR ADDRESS: Sch. Materials Science, Japan Advanced Inst. Science Technology, Tatsunokuchi, Ishikawa 923-1292, Japan**Japan
JOURNAL: Applied Microbiology and Biotechnology 50 (6): p669-675 Dec., 1998 1998
MEDIUM: print

ISSN: 0175-7598
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Protease activity was detected in the culture medium of Flavobacterium balustinum P104 grown at 10degree C, which was isolated from salmon (Oncorhynchus keta) intestine. The enzyme, designated as CP-70 protease, was purified to homogeneity from the culture broth by ion exchange and gel filtration chromatographies. The molecular mass of the protease was 70 kDa, and its isoelectric point was close to 3.5. Maximal activity toward azocasein was observed at 40degree C and from pH 7.0 to 9.0. The activity was strongly inhibited by phenylmethylsulfonyl fluoride, suggesting that the enzyme is a serine protease. The N-terminal amino acid sequence was Asp-Thr-Arg-Gln-Leu-Leu-Asn-Ala-Asn-Ser-Asp-Leu-Leu-Asn-Thr-Thr-Gly-Asn-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Leu-Thr-Gly-Ala-Phe-Asn-Gly-Glu-Asn. A search through the database for sequence homology yielded no significant match. The initial cleavage sites for oxidized insulin B-chain were found to be the Glu13-Ala14 and Phe24-Phe25 bonds. The result of the cleavage pattern of oxidized insulin B-chain suggests that CP-70 protease has a broader specificity than the other cold-active proteases against the peptide substrate.

3/7/6
DIALOG(R)File 5:Biosis Previews(R)

13144545 BIOSIS NO.: 199698612378
Characterization of a novel alpha-amylase from Lipomyces kononenkoae and expression of its gene (LKA1) in Saccharomyces cerevisiae
AUTHOR: Steyn Andries J C; Pretorius Isak S (Reprint)
AUTHOR ADDRESS: Dep. Microbiol., Inst. Wine Biotechnol., Univ. Stellenbosch, Stellenbosch 7600, South Africa**South Africa
JOURNAL: Current Genetics 28 (6): p526-533 1995 1995
ISSN: 0172-8083
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A highly active alpha-amylase (76 250 Da) secreted by the raw starch-degrading yeast Lipomyces kononenkoae strain IGC4052B was purified and characterized. Using high performance liquid chromatography (HPLC), end-product analysis indicated that the L. kononenkoae alpha-amylase acted by endo-hydrolysis on glucose polymers containing alpha-1,4 and alpha-1,6 bonds, producing mainly maltose, maltotriose and maltotetraose. The following NH-2-terminal amino acids were determined for the purified enzyme: Asp-Cys-Thr-Thr-Val-Thr-Val-Leu-Ser-Ser-Pro-Glu-Ser-%%%Val%%%-%%%Thr%%%-%%%Gly%%%. The L. kononenkoae alpha-amylase-encoding gene (LKA1), previously cloned as a cDNA fragment, was expressed in Saccharomyces cerevisiae under the control of the PGK1 promoter. The native signal sequence efficiently directed the secretion of the glycosylated protein in S. cerevisiae. De-glycosylation of the enzyme indicated that post-translational glycosylation is different in S. cerevisiae from that in L. kononenkoae. Zymogram analysis indicated that glycosylation of the protein in S. cerevisiae had a negative effect on

enzyme activity. Southern-blot analysis revealed that there is only a single LKA1 gene present in the genome of L. kononenkoae.

3/7/7
DIALOG(R)File 5:Biosis Previews(R)

12952232 BIOSIS NO.: 199598420065
Molecular interactions between fibronectin and integrins during mouse blastocyst outgrowth
AUTHOR: Yelian Frank D; Yang Yan; Hirata Janie D; Schultz Jeffery F; Armant D Randall (Reprint)
AUTHOR ADDRESS: C.S. Mott Center Human Growth Dev., Dep. Obstet. Gynecol., Wayne State Univ. Sch. Med., 275 E. Hancock Ave., Detroit, MI 48201, USA **USA
JOURNAL: Molecular Reproduction and Development 41 (4): p435-448 1995 1995
ISSN: 1040-452X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To investigate the mechanism of trophoblast adhesion to fibronectin, we cultured blastocysts in serum-free medium on proteolytic fibronectin fragments containing its major functional domains, and localized fibronectin-binding integrins in outgrowing trophoblast cells by immunofluorescent staining. Outgrowth comparable to that obtained with intact fibronectin was observed using a 120 kD chymotryptic fragment containing the central cell-binding domain (FN-120) and the Arg-Gly-Asp (RGD) recognition sequence. A 40 kD COOH-terminal chymotryptic fragment of fibronectin containing both a heparin-binding region and an alternate (non-RGD) cell-binding site was inactive in supporting trophoblast adhesion. Three synthetic peptides derived from the heparin-binding domain, including the CS1 alternate cell-binding site, were also unable to promote trophoblast cell adhesion. A 75 kD recombinant protein, ProNectin F, containing 13 copies of the cell recognition epitope of fibronectin, %%%Val%%%-%%%Thr%%%-%%%Gly%%%-Arg-Gly-Asp-Ser-Pro-Ala-Ser, vigorously supported blastocyst outgrowth. Blastocyst outgrowth was not significantly different when surfaces were precoated with cellular fibronectin, which contains an alternatively spliced type III repeat and is the form actually encountered in vivo. Several putative fibronectin receptors were localized in trophoblast outgrowths by immunofluorescent labeling. Antibodies reactive with integrin subunits alpha-3, alpha-5, alpha-IIb, alpha-v, beta-1 and beta-3, but not alpha-4, all bound to trophoblast cells. Antibodies raised against either the beta-1 or beta-3 integrin subunits significantly inhibited fibronectin-mediated outgrowth. These findings demonstrate the key role of the central cell-binding domain of fibronectin in trophoblast adhesion, and suggest four RGD-binding integrins, alpha-3-beta-1, alpha-5-beta-1, alpha-IIb-beta-3, and alpha-v-beta-3, that could mediate trophoblast adhesion in vitro and may play an important role during implantation.

3/7/8
DIALOG(R)File 5:Biosis Previews(R)

12725259 BIOSIS NO.: 199598193092
Substrate specificity and detailed characterization of a bifunctional amylase-pullulanase enzyme from Bacillus circulans F-2 having two different active sites on one polypeptide
AUTHOR: Kim Cheorl-Ho (Reprint); Kim Yong Sung
AUTHOR ADDRESS: Lab. Molecular Cellular Biology, Genetic Engineering Res. Inst., Korea Inst. Science Technol., P.O. Box 115, Yusung, Taejon 305-600, South Korea**South Korea
JOURNAL: European Journal of Biochemistry 227 (3): p687-693 1995 1995
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Bacillus circulans F-2 amylase-pullulanase enzyme (APE) displayed dual activity with respect to glycosidic bond cleavage. The enzyme was active on alpha-1,6 bonds in pullulan, amylopectin, and glycogen, while it showed alpha-1,4 activity against malto-oligosaccharides, amylose, amylopectin, and soluble starch, but not pullulan. Kinetic analysis of the purified enzyme in a system which contained both pullulan and amylose as two competing substrates was used to distinguish the dual specificity of the enzyme from the single-substrate specificity known for pullulanases and alpha-amylases. Enzyme activities were inhibited by some metal ions, and by metal-chelating agents with a different mode. The enzyme-inhibitory results of amylase and pullulanase with Hg-2+ and Co-2+ ions were different, indicating that the activation mechanisms of both enzyme activities are different. Cyclomaltoheptaose inhibited both alpha-amylase and pullulanase activities with inhibition constants (K-i) of 0.029 and 0.06 mg/ml, respectively. Modification with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide confirmed a carboxy group at the active sites of both enzymes. The N-terminal sequence of the enzyme was:
Ala-Asp-Ala-Lys-Lys-Thr-Pro-Gln-Gln-Gln-Phe-Asp-Ala-Leu-Trp-Ala-Ala-Gly-Ile-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Thr-Pro-Asp-Gly-Phe. The purified enzyme displayed Michaelis constant (K-m) values of 0.55 mg/ml for amylose, and 0.71 mg/ml for pullulan. When both amylose and pullulan were simultaneously present, the observed rate of product formation closely fitted a kinetic model in which the two substrates are hydrolyzed at different active sites. These results suggest that amylopullulanases, which possess both alpha-1,6 and alpha-1,4 cleavage activities at the same active site, should be distinguished from APEs, which contain both activities at different active sites on the same polypeptide. Also, it is proposed that the Enzyme Commission use the term 'amylase-pullulanase enzyme' to refer to enzymes which act on starch and cleave both alpha-1,6-bonds in pullulan and alpha-1,4 bonds in amylose at different active sites.

3/7/9
DIALOG(R)File 5:Biosis Previews(R)

12307594 BIOSIS NO.: 199497328879
Interdomain linkage in the polymeric hemoglobin molecule of Artemia
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JOURNAL: Journal of Molecular Evolution 38 (6): p628-636 1994 1994
ISSN: 0022-2844
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Artemia has evolved the longest known concatenation of hemoglobin domains, the alpha subunit containing nine domains and the beta subunit having a similar size. Translation of the cDNA sequence of the beta subunit reveals eight regions of inter-domain polypeptide linking together the nine heme-binding domains, together with partially analogous sequences preceding the first domain and following the last. Analysis of the structural possibilities of the linker sequences suggests how the domains may be organized in the subunit. The interdomain linker sequences were 14%-64% identical (62%-91% similar by Dayhoff substitution matrix) and approximately 14 residues in length including a consensus -Val-Asp-Pro-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Leu-. The linker composition resembled that of the 11 amino acid pre-A leader sequence of Petromyzon marinus (lamprey) hemoglobin V, the structure of which is known. Prediction of structure from the Artemia linker sequences indicated a nonhelical, turn-associated linker which could be modeled to the Petromyzon leader. Measurements confirmed that such a structure could support the packing of nine Artemia domains into a polymeric subunit of annular shape, two of which subunits (which can be similar or dissimilar) comprise the physiological molecule. The position of interdomain introns and the character of a variable residue early in the linker are compatible with the nine-domain polymer having evolved through gene duplication reflected in globin domain fusion incorporating an extension specifically of the N-terminus. The multiplication of an original single-domain globin gene to give the present nine is estimated from sequence differences, allowing for multiple mutations at individual sites, to have occurred in a period at least 500-700 million years ago.

3/7/10
DIALOG(R)File 5:Biosis Previews(R)

12208129 BIOSIS NO.: 199497229414
Water stress proteins of Nostoc commune (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1,4-beta-D-xylanxylanohydrolase activity
AUTHOR: Hill Donna R; Hladun Suzanne L; Scherer Siegfried; Potts Malcolm (Reprint)
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JOURNAL: Journal of Biological Chemistry 269 (10): p7726-7734 1994 1994
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Acidic water stress polypeptides (Wsp) with molecular masses of 33, 37 and 39 kDa are the most abundant soluble proteins in the cyanobacterium Nostoc commune. Wsp polypeptides and UV-A/B-absorbing pigments are secreted by cells, accumulate in the extracellular glycan

sheath, and are released from desiccated colonies upon rehydration. No evidence was obtained for either glycosylation, phosphorylation, or acylation of Wsp polypeptides. NH-2-terminal amino acid sequences of the 33-, 37-, and 39-kDa polypeptides were identical: Ala-Leu-Tyr-Gly-Tyr-Thr-Ile-Gly-Glu-Gln-X-Ile-Gln-Asn-Pro-Ser-Asn-Pro-Ser -Asn-Gly-Lys-Gln. This consensus NH-2-terminal sequence and an internal sequence (Glu-Ala-Arg-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Pro-Thr-Thr-Pro-Ile-Asp) showed homologies with the sequences of carbohydrate-modifying enzymes. Purified Wsp polypeptides associate with a 1,4-beta-D-xylanxylanohydrolase activity that was inhibited specifically by Wsp antiserum. In the absence of salt, Wsp polypeptides, and the water-soluble UV-A/B-absorbing pigments, form multimeric complexes through strong ionic interactions. A possible role is suggested for Wsp polypeptides in the synthesis and/or modification of a xylose-containing UV-A/B-absorbing pigment.

3/7/11
DIALOG(R)File 5:Biosis Previews(R)

12057488 BIOSIS NO.: 199497078773
The 50 kDa protein subunit of assembly polypeptide (AP) AP-2 adaptor from clathrin-coated vesicles is phosphorylated on threonine-156 by AP-1 and a soluble AP50 kinase which co-purifies with the assembly polypeptides
AUTHOR: Pauloin Alain (Reprint); Thurieau Christophe
AUTHOR ADDRESS: INRA, Laboratoire de Biologie Cellulaire Moleculaire, Centre de Recherche de Jouy, 78352 Jouy-en-Josas Cedex, France**France
JOURNAL: Biochemical Journal 296 (2): p409-415 1993 1993
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: AP50 is a subunit of the assembly polypeptide (AP) subclass AP-2 from bovine brain coated vesicles. It can be phosphorylated in vivo and in vitro on a threonine residue by means of the AP50 kinase activity associated with AP. We have undertaken an analysis of the amino acid sequence around the AP50 phosphorylation site. After phosphorylation in vitro of AP50 followed by tryptic cleavage, only one radioactive peptide was isolated following Mono-Q ion-exchange f.p.l.c. and reverse-phase h.p.l.c. The amino acid sequence of this peptide: Glu-146-Glu-Gln-Ser-Gln-Ile-Thr-Ser-Gln-%%%Val%%%-%%%Thr%%%*-%%%Gly%%% -Gln-lle-Gly-Trp-Arg-162, displayed two threonine residues. Analysis of the yield and radioactivity of the product from automated Edman degradation indicated that only Thr-156 was phosphorylated, reflecting the presence of a single phosphorylation site in AP50. AP phosphorylated the corresponding synthetic peptide on the same threonyl residue. We demonstrated that AP50 was a phosphorylation substrate unable to autophosphorylate. The enzyme involved in the AP50 phosphorylation was shown to be associated with AP-1 and with a soluble protein complex copurified with APs but resolved from the latter by hydroxyapatite-column exclusion chromatography. This AP50 kinase activity corresponded to a 280 kDa protein complex according to gel-filtration data.

3/7/12

DIALOG(R)File 5:Biosis Previews(R)

10830890 BIOSIS NO.: 199192076661
THE POLYMERIC HEMOGLOBIN MOLECULE OF ARTEMIA INTERPRETATION OF TRANSLATED COMPLEMENTARY DNA SEQUENCE OF NINE DOMAINS
AUTHOR: TROTMAN C N A (Reprint); MANNING A M; MOENS L; TATE W P
AUTHOR ADDRESS: DEP BIOCHEM, UNIV OTAGO, DUNEDIN, NEW ZEALAND**NEW ZEALAND
JOURNAL: Journal of Biological Chemistry 266 (21): p13789-13795 1991
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Translated cDNA for Artemia hemoglobin provided sequence data for almost nine domains, from the fourth residue of the A helix of one domain through 1405 residues to a stop codon after the ninth domain. The domain sequences were all different (homology between pairs 17-38%) but aligned well with each other and with conventional globins, satisfying the requirements for Phe at CD1, His at F8 and most other highly conserved features of globins including His at E7. Features found to be characteristic of Artemia globin and present in all nine domains were Phe at B10, Tyr at C4, Gly at F5, Phe at G5 and Gly at H22. Approximately 14 residues including a consensus -Val-Asp-Pro-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Leu- were available to form the linker between each pair of domains. The Artemia sequence data were compared with the crystal structures of Chironomus thummi thummi erythrocruorin III and sperm whale myoglobin in order to identify features of structural similarity and to examine the consequences of the differences. The Artemia sequences were compatible with the main helices and critical features of the globin fold. Possible modifications to the C helix, FG turn, and GH turn were studied in terms of molecular coordinates.

3/7/13
DIALOG(R)File 5:Biosis Previews(R)

09594877 BIOSIS NO.: 198987042768
RECEPTOR-MEDIATED ACTIVATION OF DETERGENT-SOLUBILIZED GUANYLATE CYCLASE
AUTHOR: BENTLEY J K (Reprint); KHATRA A S; GARBERS D L
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JOURNAL: Biology of Reproduction 39 (3): p639-648 1988
ISSN: 0006-3363
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Here for the first time we report the successful detergent-solubilization of the speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) receptor and the subsequent activation of guanylate cyclase in response to receptor occupation. Sea urchin sperm membranes treated with a solution containing 0.5% LubrolR PX and 0.5% EmulphogeneR in the presence of MgCl2 and NaF released both the speract receptor and guanylate cyclase activity into solution. The solubilized apparent receptor was not sedimented at 400,000 .times. g

.times. 15 min and was not retained by glass microfiber filters. In the presence of 125I-GGG (Y2) speract and dissuccinimidyl suberate, a major radioactive band at about Mr = 77,000 and minor bands at Mr = 35,000 and 150,000 were cross-linked. Speract but not resact (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-LeuNH2) competed in the cross-linking reaction. The amount of 125I-GG(Y2)speract bound to solubilized receptor did not increase in a linear manner as a function of added protein but instead was concave upward. The addition of speract but not react to the solublized preparation resulted in the activation of the enzyme guanylate cyclase; the extent of stimulation was dependent on the amount of enzyme protein added and also was concave upward. Approximately 900 nM speract half-maximally activated guanylate cyclase. These data suggest that the speract receptor and guanylate cyclase are closely apposed, even in detergent, or that they are the same molecule.

3/7/14
DIALOG(R)File 5:Biosis Previews(R)

09246664 BIOSIS NO.: 198886086585
COVALENT ATTACHMENT OF AN ARG-GLY-ASP SEQUENCE OF PEPTIDE TO DERIVATIZABLE POLYACRYLAMIDE SURFACES SUPPORT OF FIBROBLAST ADHESION AND LONG-TERM GROWTH
AUTHOR: BRANDLEY B K (Reprint); SCHNAAR R L
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JOURNAL: Analytical Biochemistry 172 (1): p270-278 1988
ISSN: 0003-2697
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A synthetic nonapeptide (Tyr-Ala-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Arg-Gly-Asp-Ser), which includes the adhesive Arg-Gly-Asp (RGD) sequence, was covalently immobilized on chemically well-defined polyacrylamide gel surfaces utilizing N-succinimidyl active esters. The amount of peptide immobilized varied linearly with the concentration added to the gels. Immobilization was .apprx. 80% efficient (based on peptide added), resulting in up to 1.75 nmol peptide/cm2 gel surface. Balb/c 3T3 mouse fibroblast cells adhered readily to peptide-derivatized surfaces, even in the absence of serum. Furthermore, surfaces derivatized with 2 nmol peptide/cm2 gel supported long-term fibroblast growth at a rate and to an extent comparable to that on tissue culture plastic. Surfaces derivatized with a control nonapeptide having no RGD sequence were non-supportive of cell attachment or growth. The immobilization technology used to derivatize the gel surfaces with adhesive nonapeptide can be modified to allow conderivatization with proteins, glycoproteins, glycosides, or other amine-containing compounds to test their effects on long-term cell behaviors.

3/7/15
DIALOG(R)File 5:Biosis Previews(R)

09100894 BIOSIS NO.: 198885069785

THE PRIMARY STRUCTURE OF CHICKEN MUSCLE ACYLPHOSPHATASE ISOZYME CH1
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JOURNAL: Journal of Biochemistry (Tokyo) 102 (5): p1213-1220 1987
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The amino acid sequence of chicken muscle acylphosphatase isozyme Ch1 was determined. The protein consists of 102 amino acid residues, does not contain histidine, and the NH2-terminus is acetylated: Ac-Ser-Ala-Leu-Thr-Lys-Ala-Ser-Gly-Ser-Leu-Lys-Ser-Val-Asp-Tyr-Glu-Val-Phe-Gly-Arg-Val-Gln-Gly-Val-Cys-Phe-Arg-Met-Tyr-Thr-Glu-Glu-Glu-Ala-Arg-Lys-Leu-Gly-Val-Val-Gly-Trp-Val-Lys-Asn-Thr-Ser-Gln-Gly-Thr-%%%Val%%%-%%%Thr%%%-%%%Gly%%%
-Gln-Val-Gln-Gly-Pro-Glu-Asp-Lys-Val-Asn-Ala-Met-Lys-Ser-Trp-Leu-Ser-Lys-Val-Gly-Ser-Pro-Ser-Ser-Arg-Ile-Asp-Arg-Thr-Lys-Phe-Ser-Asn-Glu-Lys-Glu-Ile-Ser-Lys-Leu-Asp-Phe-Ser-Gly-Phe-Ser-Thr-Arg-Tyr-OH. This sequence differs in 44% of the total positions from the other isozyme (Ch2) of chicken muscle acylphosphatase (Ohba et al., the accompanying paper). The sequence of Ch1 has three substitutions from that of turkey muscle acylphosphatase; these are Ser from Ala at position 9, Ser from Arg at 47, and Lys from Asn at 83. The sequence has about 80% homology with those of mammalian muscle acylphosphatases.

3/7/16
DIALOG(R)File 5:Biosis Previews(R)

09069232 BIOSIS NO.: 198885038123
RECEPTOR-MEDIATED PHOSPHORYLATION OF SPERMATOZOAN PROTEINS
AUTHOR: BENTLEY J K (Reprint); KHATRA A S; GARBERS D L
AUTHOR ADDRESS: HOWARD HUGHES MED INST, DEP PHARMACOL, VANDERBILT UNIV MED CENT, NASHVILLE, TENN 37232, USA**USA
JOURNAL: Journal of Biological Chemistry 262 (32): p15708-15713 1987
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: These studies are the first to report egg peptide-mediated stimulation of protein phosphorylation in spermatozoa. Speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) or resact (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Pro-Gly-Cys-Val-Gly-Gly-Arg-Leu-NH2) stimulated the incorporation of 32P into various proteins of isolated spermatozoan membranes in the presence, but not absence, of GTP. The Mr of three of the phosphorylated proteins were 52,000, 75,000, and 100,000. GTP-.gamma.S (guanosine 5'-O-(3-thiotriphosphate)) but not GDP.beta.S (guanosine 5'-O-(2-thiodiphosphate)) or GMP-PNP (guanylyl imidodiphosphate) also supported the peptide-mediated stimulation of protein phosphorylation. The peptides markedly stimulated guanylate cyclase activity, and GTP.gamma.S or GTP but not GMP-PNP served as effective substrates for the enzyme. The accumulation of cyclic AMP was not stimulated by the peptides. Subsequently, it was shown that added

cyclic GMP or cyclic AMP increased 32P incorporation into the same membrane proteins as those observed in the presence of peptide and GTP. The amount of cyclic GMP (up to 3 .mu.M) formed by membranes in the presence of peptide and 100 .mu.M GTP equated with the amount of added cyclic GMP required to increase the 32P content of of a Mr 75,000 protein selected for further study. 32P-Peptide maps of the Mr 75,000 protein indicated that the same domains were phosphorylated in response to cyclic nucleotides or to egg peptide and GTP. Intact cells were subsequently incubated with 32P to determine if the radiolabeled proteins observed in isolated membranes also would be obtained in intact cells. The 32P contents of proteins of Mr 52,000, 75,000, and 100,000 were significantly increased by the addition of resact. Peptide maps confirmed that the increased 32P incorporation obtained in a Mr 75,000 protein of isolated membranes occurred on the same protein domains as the 32P found on the Mr 75,000 protein of intact cells. These results suggest that a GTP or GTP.gamma.S requirement for peptide-mediated protein phosphorylation in spermatozoan membranes is mainly due to the enhanced formation of cyclic GMP, and it therefore is likely that peptide-induced elevations of cyclic nucleotide concentrations in spermatozoa are responsible for the specific increases in 32P associated with at least three sperm proteins, all apparently localized on the plasma membrane.

3/7/17
DIALOG(R)File 5:Biosis Previews(R)

08617237 BIOSIS NO.: 198783096128
SODIUM POTASSIUM ADPASE FROM PIG KIDNEYS IV. STRUCTURE OF THE ALPHA-SUBUNIT ACTIVE SITE FRAGMENT MODIFIED BY AN ALKYLATING ATP ANALOGUE
AUTHOR: DZHANDZHUGAZYAN K N (Reprint); LUTSENKO S V; MODYANOV N N
AUTHOR ADDRESS: MM SHEMYAKIN INST BIOORG CHEM, ACAD SCI USSR, MOSCOW, USSR **USSR
JOURNAL: Biologicheskie Membrany (Moscow) 3 (8): p858-868 1986
ISSN: 0233-4755
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: Covalent binding of .gamma.-[4(N-2-chloroethyl-N-methylamino)]benzylamide ATP (CLR-ATP) to the .alpha.-subunit of Na+,K+-ATPase results in drastic inhibition of ATP-hydrolysing activity. Pepsin hydrolysis of the membrane-bound Na+,K+-ATPase modified in K+-form was performed under conditions providing high stability of the modification product (4.degree. C, pH 1.5). The modified peptide was isolated by HPLC and sequenced. Its amino acid sequence Ala-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Asp-Gly-Val-Asn-Asp-Ser-Pro-Ala-Leu corresponds to region 706-718 of the .alpha.-subunit polypeptide chain. The carboxyl group of an aspartic acid residue (710 or 714) is involved in covalent binding of the reagent. This .alpha.-subunit fragment identified for the first time is a component of the enzyme active site. It is located in one of the most homologous regions revealed in catalytic subunits of all the known E1-E2 ATPases.

3/7/18
DIALOG(R)File 5:Biosis Previews(R)

08589489 BIOSIS NO.: 198783068380
AMINO ACIDS FROM TROFOPAR BY GAS-LIQUID CHROMATOGRAPHY-ELECTRON IONIZATION MASS SPECTROMETRY
AUTHOR: CULEA M (Reprint); PALIBRODA N; MERCEA V; ABRAHAM A D
AUTHOR ADDRESS: INST ISOTOPIC AND MOLECULAR TECHNOL, CLUJ-NAPOCA, ROMANIA** ROMANIA
JOURNAL: Revue Roumaine de Biochimie 23 (4): p273-278 1986
ISSN: 0001-4214
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Amino acids from Trofopar, a natural pharmaceutical product, were identified as N(O, S)-trifluoroacetylbutyl (and methyl) esters by GC/MS. The gas chromatographic separation was performed on Ethylene Glycol Adipate and OV 17. The following amino acids were found as components of Trofopar:Asp and Glu as main components and Ala, %%%Val%%%, %%%Thr%%%, %%%Gly%%%, Ser and Lys as minor components.

3/7/19
DIALOG(R)File 5:Biosis Previews(R)

08576220 BIOSIS NO.: 198783055111
COVALENT COUPLING OF A RESACT ANALOGUE TO GUANYLATE CYCLASE
AUTHOR: SHIMOMURA H (Reprint); DANGOTT L J; GARBERS D L
AUTHOR ADDRESS: DEP PHARMACOL, VANDERBILT UNIV MED CENT, NASHVILLE, TENN 37232, USA**USA
JOURNAL: Journal of Biological Chemistry 261 (33): p15778-15782 1986
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: GGGYG-resact (Gly-Gly-Gly-Tyr-Gly-Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu-NH2) was synthesized and shown to possess the same respiration-stimulationg activity and receptor-binding abilkity as resact. The incubation of intact sperm cells with radioiodinated peptide, 125I-GGGYG-resact, and the chemical cross-linking reagent, disuccinimidyl suberate, resulted in the appearance of a single, major radioactive band of apparent molecular weight 160,000 (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The interaction was specific since 150 nM nonradioactive resact but not speract (200 mM) blocked formation of the radioactive band. The radioactive, cross-linked protein co-migrated with 23P-labeled guanylate cyclase and could be immunoprecipitated with a polyclonal antibody raised in rabbits against the sperm guanylate cyclase. The incubation of intact cells with NH4Cl resulted in the partial dephosphorylation of guanylate cyclase and a change in its apparent molecular weight from 160,000 to 150,000; NH4Cl also caused the same conversion in the apparent molecular weight of the cross-linked protein. These data demonstrate that an analogue of resact can be covalently coupled to guanylate cyclase with the specificity predicted for the peptide receptor.

3/7/20
DIALOG(R)File 5:Biosis Previews(R)

08565737 BIOSIS NO.: 198783044628
DIFFERENTIAL EFFECTS OF RESACT ANALOGUES ON SPERM RESPIRATION RATES AND CYCLIC NUCLEOTIDE CONCENTRATIONS
AUTHOR: SHIMOMURA H (Reprint); GARBERS D L
AUTHOR ADDRESS: HOWARD HUGHES MED INST, VANDERBILT UNIV MED CENT, NASHVILLE, TENN 37232, USA**USA
JOURNAL: Biochemistry 25 (11): p3405-3410 1986
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Analogues of resact (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-LeuNH2) were synthesized to determine whether or not a stimulation of sperm respiration could be obtained independent of elevations of cyclic nucleotide concentrations. Modification of the CO2-terminal leucine NH2 did not alter biological activity; however, substitution of the two cysteinyl residues by Ser or Tyr or methylation of the cysteinyl residues resulted in divergent relative potencies dependent on whether respiration rates or cyclic nucleotide concentrations were measured. [Ser1,Tyr8]resact, as an example, was approximately 40% as potent as resact at stimulating respiration rates but was 1% as effective as resact at causing cyclic GMP elevations. An NH2-terminal fragment (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Ala-Pro-Gly) neither stimulated respiration nor elevated cyclic nucleotide levels at concentrations up to 10 .mu.M whereas a CO2-terminal fragment (Cys-Val-Gly-Gly-Gly-Arg-LeuNH2) had approximately 20% of the respiration activity and 0.1% of the cyclic GMP elevating activity of resact. When the CO2- and NH2-terminal fragments were added simultaneously, however, cyclic nucleotide concentrations were elevated at the same relative concentrations as observed with resact. An analogue (125I-[Tyr1,Ser8]resact) was subsequently synthesized and used for receptor binding studies. Both the NH2-terminal and CO2-terminal fragments competed for binding, although they were 0.0004 and 0.025 times as effective as resact, respectively. However, in the presence of 1 .mu.M resact-(1-7), resact-(8-14) was almost as potent as resact in the competitive binding assay. The individual CO2- and NH2-terminal fragments appear to each bind to receptor, therefore, but the presence of one greatly potentiates the binding of the other peptide fragment. It also appears that cyclic nucleotide elevations can be separated from the stimulation of respiration, suggestive that increased concentrations of cyclic AMP or cyclic GMP do not mediate the effects of the peptides on respiration.

3/7/21
DIALOG(R)File 5:Biosis Previews(R)

08559991 BIOSIS NO.: 198783038882
RECEPTOR-MEDIATED ACTIVATION OF SPERMATOZOAN GUANYLATE CYCLASE

AUTHOR: BENTLEY J K (Reprint); TUBB D J; GARBERS D L
AUTHOR ADDRESS: DEPARTMENT OF PHARMACOLOGY, VANDERBILT MEDICAL CENTER, NASHVILLE, TENNESSEE 37232, USA**USA
JOURNAL: Journal of Biological Chemistry 261 (32): p14859-14862 1986
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The sea urchin egg peptides speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) and resact (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu-NH2) bind to spermatozoa of the homologous species (Lytechinus pictus or Arbacia punctulata, respectively) and cause transient elevations of cyclic GMP concentrations (Hansbrough, J.R., and Garbers, D.L. (1981) J. Biol. Chem. 256, 1447-1452). The addition of these peptides to spermatozoan membrane preparations caused a rapid and dramatic (up to 25-fold) activation of guanylate cyclase. The peptide-induced activation of guanylate cyclase was transient, and the subsequent decline in enzyme activity coincided with conversion of a high Mr (phosphorylated) form. When membranes were incubated at pH 8.0, the high Mr form was converted to the low Mr form of guanylate cyclase was much less than the peptide-stimulated activity of the high Mr form. Activation of the low Mr form by peptide was not transient and persisted for at least 10 min. In addition, the pH 8.0 treatment that caused the Mr conversion of guanylate cyclase also caused an increase in the peptide-binding capacity of the membranes. We propose a model in which activation of the membrane form of guanylate cyclase is receptor-mediated; the extent of enzyme activation is modulated by its phosphorylation state.

3/7/22
DIALOG(R)File 5:Biosis Previews(R)

07768766 BIOSIS NO.: 198580077661
RECEPTOR-MEDIATED REGULATION OF GUANYLATE CYCLASE ACTIVITY IN SPERMATOZOA
AUTHOR: RAMARAO C S (Reprint); GARBERS D L
AUTHOR ADDRESS: HOWARD HUGHES MEDICAL INSTITUTE, VANDERBILT UNIVERSITY MEDICAL CENTER, NASHVILLE, TENN 37232, USA**USA
JOURNAL: Journal of Biological Chemistry 260 (14): p8390-8396 1985
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Two peptides, speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) and resact (Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Ala-Pro-Gly-Cys-Val-Gly-Gly-Arg-Leu-NH2), which activate sperm respiration and motility and elevate cyclic GMP concentrations in a species-specific manner, were tested for effects on guanylate cyclase activity. The guanylate cyclase of sea urchin spermatozoa is a glycoprotein and it is localized entirely on the plasma membrane. When intact sea urchin sperm cells were incubated with the appropriate peptide for time periods as short as 5 s and subsequently homogenized in detergent, guanylate cyclase activity was found to be as low as 10% of the activity of cells not treated with peptide. The peptides showed

complete species specificity and analog of 1 peptide (speract) caused decreases in enzyme activity coincident with their receptor binding properties. The peptides did not inhibit enzyme activity when added after detergent solubilization of the enzyme. When detergent-solubilized spermatozoa were incubated at 22.degree. C, guanylate cyclase activity declined in previously nontreated cells to the peptide-treated level. The rate of decline was dependent on temperature and protein concentration. When spermatozoa were 1st incubated with 32P, the decrease in guanylate cyclase activity was accompanied by a shift in the apparent MW of a major plasma membrane protein (160,000-150,000) and a loss of 32P label from the 160,000 band. Other agents (Monensin A NH4Cl) which were capable of stimulating sperm respiration and motility also caused decreases of guanylate cyclase activity when added to intact but not detergent-solubilized spermatozoa. The maximal decrease in guanylate cyclase activity occurred 5-10 min after addition of these agents. The enzyme response to Monensin A required extracellular Na+ suggestive that the ionophore caused the effect on guanylate cyclase activity by virtue of its ability to catalyze Na+/H+ exchange. Guanylate cyclase activity of sperm cells can be altered by the specific interaction of egg-associated peptides with their plasma membrane receptors.

3/7/23
DIALOG(R)File 5:Biosis Previews(R)

07757984 BIOSIS NO.: 198580066879
AMINO-ACID SEQUENCE OF ACYLPHOSPHATASE EC-3.6.1.7 FROM PORCINE SKELETAL MUSCLE
AUTHOR: MIZUNO Y (Reprint); YAMAZAKI M; TAKASAWA T; KIZAKI T; SHIOKAWA H
AUTHOR ADDRESS: SECT BIOCHEM, INST IMMUNOLOGICAL SCI, HOKKAIDO UNIV, KITA-KU, SAPPORO, HOKKAIDO 060**JAPAN
JOURNAL: Journal of Biochemistry (Tokyo) 97 (4): p1135-1142 1985
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The amino acid sequence of acylphosphatase from porcine skeletal muscle was determined. It consists of 98 amino acid residues with N-acetylserine at the amino (N)-terminus:
Ac-Ser-Thr-Ala-Arg-Pro-Leu-Lys-Ser-Val-Asp-Tyr-Glu-Val-Phe-Gly-Arg-Val-Gln-Gly-Val-Cys-Phe-Arg-Met-Tyr-Thr-Glu-Asp-Glu-Ala-Arg-Lys-Ile-Gly-Val-Val-Gly-Trp-Val-Lys-Asn-Thr-Ser-Lys-Gly-Thr-%%%Val%%%-%%%Thr%%%-%%%Gly%%%-Gln-Val-Gln-Gly-Pro-Glu-Glu-Lys-Val-Asn-Ser-Met-Lys-Ser-Trp-Leu-Ser-Lys-Ile-Gly-Ser-Pro-Ser-Ser-Arg-Ile-Asp-Arg-Thr-Asn-Phe-Ser-Asn-Glu-Lys-Thr-Ile-Ser-Lys-Leu-Glu-Tyr-Ser-Asn-Phe-Ser-Ile-Arg-Tyr-OH. This sequence has 3 substitutions of amino acid residues,.i.e., Thr/Ala, Ile/Val, and Ile/Val at positions 26, 68 and 96, respectively, from that of horse muscle acylphosphatase, formerly the only mammalian acylphosphatase with known sequence.

3/7/24
DIALOG(R)File 5:Biosis Previews(R)

07649690 BIOSIS NO.: 198579068589
A PEPTIDE ASSOCIATED WITH EGGS CAUSES A MOBILITY SHIFT IN A MAJOR PLASMA MEMBRANE PROTEIN OF SPERMATOZOA
AUTHOR: SUZUKI N (Reprint); SHIMOMURA H; RADANY E W; RAMARAO C S; WARD G E; BENTLEY J K; GARBERS D L
AUTHOR ADDRESS: HOWARD HUGHES MED INST, DEP PHARMACOLOGY, VANDERBILT UNIV MED CENTER, NASHVILLE, TENNESSEE 37232, USA**USA
JOURNAL: Journal of Biological Chemistry 259 (23): p14874-14879 1984
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A peptide (resact) associated with the eggs of the sea urchin, Arbacia punctulata, which stimulates sperm respiration rates by 5- to 10-fold, was purified and its amino acid sequence was determined. The sequence was found to be Cys-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu-NH2. The peptide was subsequently synthesized by solid phase methods, amidated at the carboxyl-terminal Leu, and shown to be identical to the isolated, native material. The peptide half-maximally stimulated A. punctulata spermatozoan respiration at 0.5 nM and half-maximally elevated cGMP concentrations at 25 nM at an extracellular pH of 6.6. The increase in O2 consumption was coupled with a stimulation of motility. However, at elevated extracellular pH (pH 8.0), resact failed to appreciably stimulate respiration while the elevations of cGMP continued to occur. Resact did not cross-react with sperm cells obtained from Lytechinus pictus or Strongylocentrotus purpuratus; a peptide (speract) obtained from S. purpuratus eggs (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) which activates S. purpuratus sperm respiration did not stimulate A. punctulata spermatozoa. Resact caused a shift in the apparent MW (160,000-150,000) of a major sperm plasma membrane protein; as with cGMP elevations, this response was evident at extracellular pH values of both 6.6 and 8.0. The protein exists in the cell as a phosphoprotein and 32P is released coincident with the MW change. Approximately 115 nM resact caused one-half-maximal conversion of the 160,000-dalton protein after 1 min of incubation. Resact caused the apparent MW conversion of the protein within 5 s and appeared to do so in an irreversible manner. The MW change of the protein was also observed after the addition of monensin A (25 .mu.M) and NH4Cl (40 mM), 2 agents known to elevate intracellular pH and to increase sperm respiration rates. The membrane protein appears to be the enzyme guanylate cyclase, but since concentrations of resact causing one-half-maximal conversion of the MW = 160,000 form of the enzyme are about 250 times higher than those causing one-half-maximal stimulation of respiration, the relationship of the apparent molecular weight conversion to a subsequent physiological event remains unclear.

3/7/25
DIALOG(R)File 5:Biosis Previews(R)

07278645 BIOSIS NO.: 198478014052
AMINO-ACID SEQUENCE OF THE K PEPTIDE GENERATED BY LIMITED PROTEOLYSIS OF GLUCOSE DEHYDROGENASE EC-1.1.1.47 FROM BACILLUS-MEGATERIUM BY PROTEINASE K
AUTHOR: JANY K-D (Reprint); NITSCHE E

AUTHOR ADDRESS: INSTITUT FUER ORGANISCHE CHEMIE, BIOCEHMIE, UND ISOTOPENFORSCHUNG, UNIV STUTTGART, PFAFFENWALDRING 55, D-7000 STUTTGART 80, WEST GERMANY**WEST GERMANY
JOURNAL: Archives of Biochemistry and Biophysics 229 (1): p355-358 1984
ISSN: 0003-9861
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Proteolysis of glucose dehydrogenase from B. megaterium with proteinase K apparently generated 2 fragments. The small fragment, designated as K-peptide, was sequenced and its covalent structure was determined as Ser-Ser-Glu-Ala-Ser-Tyr-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Ile-Thr-Leu-Phe-Ala-Asp-Gly-Gly-Met-Thr-Gln-Tyr-Pro-Ser-Phe-Glu-Ala-Gly-Arg-Gly. The sequence analysis showed that the K-peptide consists of 2 identical fragments, one of which is lacking 1 serine residue at the amino-terminus.

06598478 BIOSIS NO.: 198274014901
AMINO TERMINAL SEQUENCES OF DNA BINDING HEPARIN BINDING AND GELATIN BINDING TRYPTIC FRAGMENTS FROM HUMAN PLASMA FIBRONECTIN
AUTHOR: PANDE H (Reprint); SHIVELY J E
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JOURNAL: Archives of Biochemistry and Biophysics 213 (1): p258-265 1982
ISSN: 0003-9861
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: NH2-terminal sequence analysis was performed on subregions of human plasma fibronectin including the 24,000 dalton (24K) DNA-binding fragment the 29,000 dalton (29K) gelatin-binding fragment and 18,000 dalton (18K) heparin-binding tryptic fragments. These fragments were obtained from fibronectin after extensive trypsin digestion followed by sequential affinity purification on gelatin-Sepharose, heparin-agarose and DNA-cellulose columns. The gelatin-binding fragment was further purified by gel filtration on Sephadex G-100, and the DNA-binding and heparin-binding fragments were further purified by high-performance liquid chromatography. The 29K fragment had the following NH2-terminal sequence:
Ala-Ala-Val-Tyr-Gln-Pro-Gln-Pro-His-Pro-Gln-Pro-Pro-(Pro)-Tyr-Gly-His-His -Val-Thr-Asp-(His)-(Thr)-Val-Val-Tyr-Gly-(Ser)-?-(Ser)-?-Lys. The NH2-terminal sequence of a 50K, gelatin-binding, subtilisin fragment (Gold, L.I. et al., 1979) is identical to positions 3-19 (with the exception of some ambiguity at position 14) of the 29K fragment. The 29K tryptic fragment may be included in the 50K subtilisin fragment, and subtilisin may cleave fibronetin between the Ala2-Val3 residues of the 29K tryptic fragment. The 18K heparin-binding fragment had the following NH2-terminal sequence:
(Glu)-Ala-Pro-Gln-Pro-His-Cys-Ile-Ser-Lys-Tyr-Ile-Leu-Tyr-Trp-Asp-Pro-Lys -Asn-Ser-Val-Gly-?-(Pro)-Lys-Glu-Ala-?-(Val)-(Pro). The 29K

gelatin-binding and 18K heparin-binding fragments have proline-rich NH2-terminal sequences, suggesting that they may have arisen from protease-sensitive, random coil regions of fibronectin corresponding to interdomain regions preceding macromolecular-binding domains. Both of these fragments contain the identical sequence Pro-Gln-Pro-His, a sequence which may be repeated in other interdomain regions of fibronectin. The 24K DNA-binding fragment has the following NH2-terminal sequence:
Ser-Asp-Thr-Val-Pro-Ser-Pro-Cys-Asp-Leu-Gln-Phe-Val-Glu-Val-Thr-Asp-Val-Lys-Val-Thr-Ile-Met-Trp-Thr-Pro-Pro-Glu-Ser-Ala-%%%Val%%%-%%%Thr%%%-%%%Gly%%%
-Tyr-Arg-Val-Asp-Val-Cys-Pro-Val-Asn-Leu-Pro-Gly-Glu-His-Gly-Gln-(Cys)-Leu-Pro-Ile-Ser. The sequence of positions 9-22 are homologous to positions 15-28 of the .alpha. chain of DNA-dependent RNA polymerase Escherichia coli. The homology observed suggests that this stretch of amino acids may be a DNA-binding site.

3/7/27
DIALOG(R)File 5:Biosis Previews(R)

06550138 BIOSIS NO.: 198273054065
ISOLATION AND SEQUENCE ANALYSIS OF THE INTRA MEMBRANOUS HYDROPHOBIC SEGMENT OF THE H-2K-B MURINE HISTO COMPATIBILITY ANTIGEN
AUTHOR: UEHARA H (Reprint); COLIGAN J E; NATHENSON S G
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JOURNAL: Biochemistry 20 (21): p5936-5939 1981
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The primary structure of the intramembranous segment (TC-1) of the mouse transplantation antigen, H-2Kb, was determined. The segment contains a stretch of 31 uncharged amino acid residues and is localized between the NH2-terminal and the COOH-terminal hydrophilic regions of the molecule. The amino acid sequence of TC-1 is
Trp-Asp-Glu-Pro-Pro-Ser-Thr-Val-Ser-Asn-Met-Ala-Thr-Val-Ala-Val-Leu-Val-Val-Leu-Gly-Ala-Ala-Ile-%%%Val%%%-%%%Thr%%%-%%%Gly%%%
-Ala-Val-Val-Ala-Phe-Val-Met-Lys-Met-Arg-(Arg)-(Arg).

3/7/28
DIALOG(R)File 5:Biosis Previews(R)

05406010 BIOSIS NO.: 197865066997
THE PYRIDOXAL BINDING SITE IN PYRIDOXAMINE PYRUVATE TRANS AMINASE EC-2.6.1.30
AUTHOR: HODSDON J (Reprint); KOLB H; SNELL E E; COLE R D
AUTHOR ADDRESS: DEP BIOCHEM, UNIV CALIF, BERKELEY, CALIF 94720, USA**USA
JOURNAL: Biochemical Journal 169 (2): p429-432 1978
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: The enzyme-substrate complex formed between pyridoxamine-pyruvate transaminase (EC 2.6.1.30) [from Pseudomonas] and pyridoxal was reduced with NaBH4. After carboxymethylation and tryptic digestion, pyridoxyl[Pxy]-lysine-containing peptides were isolated by a combination of Sephadex and Dowex 50 chromatography. Analysis of these peptides shows the structure around the pyridoxal-binding lysine residues to be Ala-Asp-Ile-Tyr-%%%Val%%%-%%%Thr%%%-%%%Gly%%% -Pro-Adx-Lys(Pxy)-Cys-Leu(Pro2,Gly2Ala2, Met)(Thr,Leu2)Gly-Val-Ser-Glu-Arg. This structure differs from those found for the corresponding peptides from pyridoxal phosphate-dependent enzymes.

```
? s (Val()Thr()Gly()Glu) and hirudin?
          15853  VAL
          15212  THR
          24858  GLY
          21271  GLU
              0  VAL(W)THR(W)GLY(W)GLU
           9454  HIRUDIN?
      S4       0  (VAL()THR()GLY()GLU) AND HIRUDIN?
? s (val()thr()gly()glu)
          15853  VAL
          15212  THR
          24858  GLY
          21271  GLU
      S5       0  (VAL()THR()GLY()GLU)
? s VTGE
      S6       0  VTGE
? S (Thr()Pro()lys) and hirudin
          15212  THR
          76406  PRO
          20182  LYS
             28  THR(W)PRO(W)LYS
           2990  HIRUDIN
      S7       1  (THR()PRO()LYS) AND HIRUDIN
? t s9/7/1

 S9/7/1
>>>Set 9 does not exist
? t s7/7/1

 7/7/1
DIALOG(R)File   5:Biosis Previews(R)
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11287775   BIOSIS NO.: 199293130666
%%%HIRUDIN%%% C-TERMINAL FRAGMENTS INHIBIT THROMBIN INDUCED NEUTROPHIL
  CHEMOTAXIS
AUTHOR: ROWAND J K (Reprint); MARUCHA P; BERLINER L J
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  COLUMBUS, OHIO 43210-1173, USA**USA
JOURNAL: Thrombosis and Haemostasis  67 (3): p289-291 1992
ISSN: 0340-6245
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH
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ABSTRACT: Since native %%%hirudin%%% blocks the thrombin induced chemotaxis response of neutrophils, we examined whether %%%hirudin%%% C-terminal peptides were also capable of this inhibition. The studies showed that thrombin induced human neutrophil chemotaxis was effectively blocked by the C-terminal %%%hirudin%%% peptide analogs, Gly-Asp-Phe-GLu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln (12-mer[54-65]) and %%%Thr%%%-%%%Pro%%%-%%%Lys%%% -Pro-Gln-Ser-His-Asn-Asp-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln (21-mer[45-65]). Furthermore, neither peptide had an effect on formyl-L-methionyl-L-leucyl-L-phenylalanine induced chemotaxis. The results suggest that binding of the %%%hirudin%%% C-terminal peptides block the thrombin chemotactic domain.

```
? s (ser()asp()gly) and hirudin?
          25316  SER
          21589  ASP
          24858  GLY
             30  SER(W)ASP(W)GLY
           9454  HIRUDIN?
     S8       1  (SER()ASP()GLY) AND HIRUDIN?
? t s8/7/1
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 8/7/1
DIALOG(R)File   5:Biosis Previews(R)
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11775330 BIOSIS NO.: 199395077596
Hirudisins: %%%Hirudin%%%-derived thrombin inhibitors with disintegrin activity
AUTHOR: Knapp Axel; Degenhardt Thorsten; Dodt Johannes (Reprint)
AUTHOR ADDRESS: Institut Biochemie, Technische Hochschule Darmstadt, Petersenstrasse 22, D-6100 Darmstadt, Germany**Germany
JOURNAL: Journal of Biological Chemistry 267 (34): p24230-24234 1992
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Recombinant %%%hirudin%%% variants have been designed which inhibit alpha-thrombin by the %%%hirudin%%% mechanism and which in addition exhibit disintegrin activity. These proteins, called "hirudisins," have been engineered by replacing the %%%Ser%%%-%%%Asp%%%-%%%Gly%%%-Glu sequence at the tip of %%%hirudin%%%'s finger-like structure (residues 32-35) by Arg-Gly-Asp-Ser (RGDS) to yield hirudisin and Lys-Gly-Asp-Ser (KGDS) to obtain hirudisin-1. Comparison of thrombin inhibition activities showed that hirudisin is 2-fold more potent (K-i = 160 +- 70 fM) than hirudisin-1 (K-i = 370 +- 44 fM) and recombinant (r)-%%%hirudin%%% (K-i = 270 +- 50 fM). alpha-Thrombin-stimulated platelet aggregation was effectively inhibited by r-%%%hirudin%%%, hirudisin, and hirudisin-1 with IC-50 of 5.7 to 6.8 nM. Unlike r-%%%hirudin%%%, hirudisin inhibits ADP-induced platelet aggregation (IC-50 = 65 mu-M) 3- to 5-fold stronger than the linear GRGDS- and RGDS-peptide. Direct interaction of hirudisin with purified glycoprotein IIb-IIIa demonstrated that antiplatelet aggregation activity is due to the integrin-directed RGD motif. Disintegrin activity of hirudisin relative to that of reduced and carboxymethylated hirudisin suggests that the conformational strain favors binding to integrins. On the basis of these results, hirudisins

appear to be interesting molecules for the design of potential antithrombotic agents with antithrombin as well as antiplatelet aggregation activities.

```
? s (hirudin?()peptide?) and ((amino()terminal) or (N()terminal))
           9454  HIRUDIN?
         375450  PEPTIDE?
             36  HIRUDIN?(W)PEPTIDE?
         594046  AMINO
         272830  TERMINAL
          29139  AMINO(W)TERMINAL
        1318531  N
         272830  TERMINAL
          73196  N(W)TERMINAL
     S9       2  (HIRUDIN?()PEPTIDE?) AND ((AMINO()TERMINAL) OR
                 (N()TERMINAL))
? t s9/7/1-2

 9/7/1
DIALOG(R)File   5:Biosis Previews(R)
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13591780 BIOSIS NO.: 199699225840

Identification and characterization of the thrombin binding sites on fibrin

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JOURNAL: Journal of Biological Chemistry 271 (38): p23121-23125 1996 1996

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Thrombin binds to fibrin at two classes of non-substrate sites, one of high affinity and the other of low affinity. We investigated the location of these thrombin binding sites by assessing the binding of thrombin to fibrin lacking or containing gamma' chains, which are fibrinogen gamma chain variants that contain a highly anionic carboxyl-terminal sequence. We found the high affinity thrombin binding site to be located exclusively in D domains on gamma' chains (K-a, 4.9 times 10-6 M-1; n, 1.05 per gamma' chain), whereas the low affinity thrombin binding site was in the fibrin E domain (K-a, 0.29 times 10-6 M-1; n, 1.69 per molecule). The %%%amino%%%-%%%terminal%%% beta-15-42 fibrin sequence is an important constituent of low affinity binding, since thrombin binding at this site is greatly diminished in fibrin molecules lacking this sequence. The tyrosine-sulfated, thrombin exosite-binding %%%hirudin%%% %%%peptide%%%, S-Hir-53-64 (hirugen), inhibited both low and high affinity thrombin binding to fibrin (IC-50 1.4 and 3.0 mu-M, respectively). The presence of the high affinity gamma' chain site on fibrinogen molecules did not inhibit fibrinogen conversion to fibrin as assessed by thrombin time measurements, and thrombin exosite binding to fibrin at either site did not inhibit its catalytic activity toward a small thrombin substrate, S-2238. We infer from these findings that there are two low affinity non-substrate thrombin binding sites, one in each half of the dimeric fibrin E domain, and that they may represent a residual aspect of thrombin binding and cleavage of its substrate fibrinogen. The high affinity thrombin binding site on gamma' chains is a constitutive feature of fibrin as well as fibrinogen.

9/7/2
DIALOG(R)File 5:Biosis Previews(R)

11158414 BIOSIS NO.: 199293001305
THE %%%AMINO%%%-%%%TERMINAL%%% ACIDIC DOMAIN OF HEPARIN COFACTOR II MEDIATES THE INHIBITION OF ALPHA THROMBIN IN THE PRESENCE OF GLYCOSAMINOGLYCANS
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JOURNAL: Journal of Biological Chemistry 266 (30): p20223-20231 1991
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Heparin cofactor II (HCII) is a glycoprotein in human plasma that inhibits thrombin and chymotrypsin. Inhibition occurs when the protease attack the reactive site peptide bond in HCII (Leu444-Ser445) and becomes trapped as a covalent 1:1 complex. Dermatan sulfate and heparin increase the rate of inhibition of thrombin, but not of chymotrypsin, > 1000-fold. The %%%N%%%-%%%terminal%%% portion of HCII contains two acidic repeats (Glu56-Asp-Asp-Asp-Tyr-Leu-Asp and Glu69-Asp-Asp-Asp-Tyr-Ile-Asp) that may bind to anion-binding exosite I of thrombin to facilitate covalent complex formation. To examine the importance of the acidic domain, we have constructed a series of 5' deletions in the HCII cDNA and expressed the recombinant HCII (rHCII) in Escherichia coli. Apparent second-order rate constants (k2) for inhibition of .alpha.-thrombin and chymotrypsin by each variant were determined. Deletion of amino acid residues 1-74 had no effect on the rate of inhibition of .alpha.-thrombin or chymotrypsin in the absence of a glycosaminoglycan. Similarly, the rate of inhibition of .alpha.-thrombin in the presence of a glycosaminoglycan was unaffected by deletion of residues 1-52. However, deletion of residues 1-67 (first acidic repeat) or 1-74 (first and second acidic repeats) greatly decreased the rate of inhibition of .alpha.-thrombin in the presence of heparin, dermatan sulfate, or a dermatan sulfate hexasaccharide that comprises the minimum high-affinity binding site for HCII. Deletion of one or both of the acidic repeats increased the apparent affinity of rHCII for heparin-Sepharose, suggesting that the acidic domain may interact with the glycosaminoglycan-binding site of native rHCII. The stimulatory effect of glycosaminoglycans on native rHCII was decreased by a C-terminal %%%hirudin%%% %%%peptide%%% which binds to anion-binding exosite I of .alpha.-thrombin. Furthermore, the ability of native rHCII to inhibit .gamma.-thrombin, which lacks the binding site for hirudin, was stimulated weakly by glycosaminoglycans. These results support a model in which the stimulatory effect of glycosaminoglycans on the inhibition of .alpha.-thrombin is mediated, in part, by the %%%N%%%-%%%terminal%%% acidic domain of HCII.
? s hirugen
S10 63 HIRUGEN
? t s10/7/60-63

10/7/60
DIALOG(R)File 5:Biosis Previews(R)

10653437 BIOSIS NO.: 199191036328
THROMBIN'S ENZYMATIC ACTIVITY INCREASES PERMEABILITY OF ENDOTHELIAL CELL MONOLAYERS
AUTHOR: DEMICHELE M A A (Reprint); MOON D G; FENTON J W II; MINNEAR F L
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JOURNAL: Journal of Applied Physiology 69 (5): p1599-1606 1990
ISSN: 8750-7587
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Human .alpha.-thrombin increases the permeability of bovine pulmonary artery endothelial cell (CCL-209) monolayers. To determine if this increase is via an enzymatic or receptor-mediated mechanism, enzymatically active forms of .alpha.-thrombin and enzymatically inactive forms with cell binding activity were incubated with the monolayers. Enzymatic forms included .alpha.-thrombin and two digestion products, .zeta.-thrombin (chymotryptic product with 89% clotting activity) and .gamma.-thrombin (tryptic product). Enzymatically inactive forms included D-Phe-Pro-Arg-chloromethylketone-(PPACK) .alpha.-thrombin and diisopropylphosphorofluoridate-CDIP .alpha.-thrombin. Cell binding activity of .alpha.- and PPACK-.alpha.-thrombin was demonstrated to be similar to each other and comparable to that cited in the literature for DIP-.alpha.-thrombin. .gamma.-Thrombin, on the other hand, did not compete for binding of 125I-labeled .alpha.-thrombin. All enzymatic forms of .alpha.-thrombin increased endothelial permeability as assessed by the clearance of 125I-albumin across the monolayers. Coincubation of PPACK, an enzymatic site inhibitor, with .alpha.- or .gamma.-thrombin prevented the increase in permeability, further indicating that .alpha.-thrombin increased permeability, by its enzymatic activity. Both enzymatically inactive forms of .alpha.-thrombin with high-affinity binding activity had no effect on permeability. To further examine whether cell binding activity of .alpha.-thrombin contributed to the increased permeability, a sulfated COOH-terminal fragment of hirudin (%%%hirugen%%%) that binds to the anion-binding site of .alpha.-thrombin but, unlike hirudin, does not interact with the catalytic site was conincubated with .alpha.-thrombin. Addition of %%%hirugen%%% (which inhibited binding of 125I-.alpha.-thrombin comparable to competition of 125I-.alpha.-thrombin with unlabeled .alpha.- or PPACK-.alpha.-thrombin) to .alpha.-thrombin did not prevent the increase in permeability. These findings indicate that the thrombin-induced increase in endothelial permeability requires thrombin's enzymatic activity but not high-affinity binding activity.

10/7/61
DIALOG(R)File 5:Biosis Previews(R)

10318266 BIOSIS NO.: 199090102745
THE CARBOXYL-TERMINAL DOMAIN OF HIRUDIN AN EXOSITE-DIRECTED COMPETITIVE INHIBITOR OF THE ACTION OF ALPHA THROMBIN ON FIBRINOGEN
AUTHOR: NASKI M C (Reprint); FENTON J W II; MARAGANORE J M; OLSON S T; SHAFER J A
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**USA
JOURNAL: Journal of Biological Chemistry 265 (23): p13484-13489 1990
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Hirudin, a potent 65-residue polypeptide inhibitor of .alpha.-thrombin found in the saliva of the leech Hirudo medicinalis, and fragments thereof are potentially useful as antithrombotic agents. %%%Hirugen%%%, the synthetic N-acetylated COOH-terminal dodecapeptide (Ac-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr(SO3)-Leu) of hirudin was shown in the present study to behave as a pure competitive inhibitor (Ki = 0.54 .mu.M) of human .alpha.-thrombin-catalyzed release of fibrinopeptide A from human fibrinogen. In contrast to this inhibitory activity, %%%hirugen%%% slightly enhanced (increased kcat/Km 1.6-fold) .alpha.-thrombin-catalyzed hydrolysis of the fluorogenic tripeptide substrate N-p-Tosyl-Gly-Pro-Arg-7-amido-4-methylcoumarin. These observations indicate that %%%hirugen%%% binds to .alpha.-thrombin at an exosite distinct from the active site, and that interaction with this exosite is a major determinant of the competence of .alpha.-thrombin to bind fibrinogen. Consistent with this view, %%%hirugen%%% blocked binding of fibrin II to .alpha.-thrombin. Studies of the effect of %%%hirugen%%% on the rate of inactivation of .alpha.-thrombin by antithrombin III (AT), the major plasma inhibitor of .alpha.-thrombin, indicated that binding of %%%hirugen%%% to .alpha.-thrombin results in less than a 2.5-fold decrease in the rate of inactivation of .alpha.-thrombin by AT, both in the absence and presence of heparin. This behavior is distinct from that of active site-directed competitive inhibitors of .alpha.-thrombin which bind to .alpha.-thrombin and block both conversion of fibrinogen to fibrin and inactivation of .alpha.-thrombin by AT. %%%Hirugen%%%, an exosite-directed competitive inhibitor, blocks the interaction of .alpha.-thrombin with fibronogen while leaving .alpha.-thrombin competent to react with AT. Thus, unlike active site-directed competitive inhibitors, %%%hirugen%%% should act in concert with AT and heparin to reduce the amount of fibrinogen that is processed during the lifetime of .alpha.-thrombin in plasma.

10/7/62

10310771 BIOSIS NO.: 199090095250
CLOT-BOUND THROMBIN IS PROTECTED FROM INHIBITION BY HEPARIN ANTITHROMBIN III BUT IS SUSCEPTIBLE TO INACTIVATION BY ANTITHROMBIN III-INDEPENDENT INHIBITORS
AUTHOR: WEITZ J I (Reprint); HUDOBA M; MASSEL D; MARAGANORE J; HIRSH J
AUTHOR ADDRESS: HENDERSON GENERAL HOSP, 711 CONCESSION ST, HAMILTON, ONTARIO L8V 1C3, CAN**CANADA
JOURNAL: Journal of Clinical Investigation 86 (2): p385-391 1990
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Propagation of venous thrombi or rethrombosis after coronary

thrombolytic therapy can occur despite heparin administration. To explore potential mechanisms, we set out to determine whether clot-bound thrombin is relatively protected from inhibition by heparin-antithrombin III but susceptible to inactivation by antithrombin III-independent inhibitors. Using plasma fibrinopeptide A (FPA) levels as an index of thrombin activity, we compared the ability of thrombin inhibitors to block FPA release mediated by fluid-phase thrombin with their activity against the clot-bound enzyme. Incubation of thrombin with citrated plasma results in concentration-dependent FPA generation, which reaches a plateau within minutes. In contrast, there is progressive FPA generation when fibrin clots are incubated with citrated plasma. Heparin, hirudin, hirudin dodecapeptide (%%%hirugen%%%), and D-phenylalanyl-L-prolyl-L-arginyl chloromethyl ketone (PPACK) produce concentration-dependent inhibition of FPA release mediated by fluid-phase thrombin. However, heparin is much less effective at inhibiting thrombin bound to fibrin because a 20-fold higher concentration is necessary to block 70% of the activity of the clot-bound enzyme than is required for equivalent inhibition of fluid-phase thrombin (2.0 and 0.1 U/ml, respectively). In contrast, %%%hirugen%%% and PPACK are equally effective inhibitors of fluid- and solid-phase thrombin, while hirudin is only 50% as effective against the clot-bound enzyme. None of the inhibitors displace bound 125I-labeled thrombin from the clot. These studies indicate tht (a) clot-bound thrombin is relatively protected from inhibition by heparin, possibly because the heparin binding site on thrombin is inaccessilble when the enzyme is bound to fibrin, and (b) clot-bound thrombin is susceptible to inactivation by antithrombin III-independent inhibitors because the sites of their interaction are not masked by thrombin binding to fibrin. For these reasons, antithrombin III-independent inhibitors may be more effective than heparin in certain clinical settings.

10080082 BIOSIS NO.: 199039133471
PREVENTION OF RETHROMBOSIS WITH %%%HIRUGEN%%% IN A DOG MODEL OF ARTERIAL THROMBOSIS
AUTHOR: BADYLAK S F (Reprint); VOYTIK S; HENKIN J; BURKE S
AUTHOR ADDRESS: PURDUE UNIV, WEST LAFAYETTE, INDIANA, USA**USA
JOURNAL: Fibrinolysis 4 (SUPPL. 3): p9 1990
CONFERENCE/MEETING: TENTH INTERNATIONAL CONGRESS ON FIBRINOLYSIS, INDIANAPOLIS, INDIANA, USA, AUGUST 4-8, 1990. FIBRINOLYSIS.
ISSN: 0268-9499
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
? s hirugensin?
S11 0 HIRUGENSIN?
? s hirugisin?
S12 0 HIRUGISIN?
? ds

Set	Items	Description
S1	70	HIRUDIN?(2W)PEPTIDE?
S2	0	(VAL()THR()GLY) AND(HIRUDIN?(2W)PEPTIDE?)
S3	28	(VAL()THR()GLY)

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S4            0    (VAL()THR()GLY()GLU) AND HIRUDIN?
S5            0    (VAL()THR()GLY()GLU)
S6            0    VTGE
S7            1    (THR()PRO()LYS) AND HIRUDIN
S8            1    (SER()ASP()GLY) AND HIRUDIN?
S9            2    (HIRUDIN?()PEPTIDE?) AND ((AMINO()TERMINAL) OR (N()TERMINA-
                L))
S10          63    HIRUGEN
S11           0    HIRUGENSIN?
S12           0    HIRUGISIN?
? log y
       12sep07 07:59:55 User217744 Session D1071.3
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             $131.10  57 Type(s) in Format  7
          $131.10  57 Types
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     $4.80  TELNET
   $154.90  Estimated cost this search
   $154.92  Estimated total session cost   3.550 DialUnits
Logoff: level 05.19.02 D  07:59:55
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File 5:Biosis Previews(R) 1926-2007/Nov W2
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Nov '07

2004

Set	Items	Description
? s hirudin? and fragment?		
	9497	HIRUDIN?
	277417	FRAGMENT?
S1	299	HIRUDIN? AND FRAGMENT?
? s s1 and trypsin		
	299	S1
	61777	TRYPSIN
S2	22	S1 AND TRYPSIN

? t s2/7/1-22

2/7/1
DIALOG(R)File 5:Biosis Previews(R)

16354670 BIOSIS NO.: 200100526509
Domain and genomic sequence analysis of bdellin-KL, a leech-derived %%%trypsin%%%-plasmin inhibitor
AUTHOR: Kim Young Hye; Choi Jung Gyoon; Lee Gyun Min; Kang Ke Won (Reprint)
AUTHOR ADDRESS: Bioresource Laboratory, Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon, 305-701, South Korea**South Korea
JOURNAL: Journal of Biochemistry (Tokyo) 130 (3): p431-438 Sep., 2001 2001
MEDIUM: print
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Bdellin-KL is a %%%trypsin%%%-plasmin inhibitor from Hirudo nipponia, whose N-terminal sequence was identified as a non-classical Kazal-type. A cDNA clone encoding the inhibitor was isolated by reverse transcription-PCR and 5' rapid amplification of cDNA ends. The cDNA showed an open reading frame of 155 amino acids comprising one signal peptide and two separated domains. The C-terminal domain consists of distinct internal repeats, including HHEE and HHDD. The bdellin-KL sequence, from the constructed genomic library of Korean leech, was determined for the 2109 bases comprising the open reading frame and flanking regions (3' and 5'). The promoter region contains potential regulatory sequence motifs, including TATA, CAAT, and GC boxes. To characterize the properties of each domain, an N-terminal %%%fragment%%% was prepared by limited proteolysis of the intact protein. The inhibitory activity of the region was as potent as that of the intact protein. This suggests that the compact domain plays an important part in the inhibitory action of bdellin-KL. The C-terminal domain was revealed to have binding affinity to ions such as Ca2+, Zn2+, Fe3+, and Fe2+ without an influence on the inhibitory activity. This study demonstrates that bdellin-KL may be a novel bifunctional protein with two distinct domains.

2/7/2
DIALOG(R)File 5:Biosis Previews(R)

16117630 BIOSIS NO.: 200100289469
Application of genetic semihomology algorithm to theoretical studies on various protein families
AUTHOR: Leluk Jacek (Reprint); Hanus-Lorenz Beata; Sikorski Aleksander F
AUTHOR ADDRESS: Institute of Biochemistry and Molecular Biology, University of Wroclaw, Tamka 2, 50-137, Wroclaw, Poland**Poland
JOURNAL: Acta Biochimica Polonica 48 (1): p21-33 2001 2001
MEDIUM: print
ISSN: 0001-527X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Several protein families of different nature were studied for genetic relationship, correct alignment at non-homologous %%%fragments%%% , optimal sequence consensus construction, and confirmation of their actual relevance. A comparison of the genetic semihomology approach with statistical approaches indicates a high accuracy and cognition significance of the former. This is particularly pronounced in the study of related proteins that show a low degree of homology. The sequence multiple alignments were verified and corrected with respect to the questionable, non-homologous %%%fragments%%%. The verified alignments were the basis for consensus sequence formation. The frequency of six-codon amino acids occurrence versus position variability was studied and their possible role in amino acid mutational exchange at variable positions is discussed.

2/7/3
DIALOG(R)File 5:Biosis Previews(R)

15781161 BIOSIS NO.: 200000499474
New bivalent thrombin inhibitors with Nalpha(methyl)arginine at the P1-position
AUTHOR: Steinmetzer Torsten (Reprint); Batdordshjin Mjanganzezeg; Pineda Felipe; Seyfarth Lydia; Vogel Andre; Reissmann Siegmund; Hauptmann Joerg; Stuerzebecher Joerg
AUTHOR ADDRESS: Haemosys GmbH, Drackendorfer Str. 1, D-07747, Jena, Germany **Germany
JOURNAL: Biological Chemistry 381 (7): p603-610 July, 2000 2000
MEDIUM: print
ISSN: 1431-6730
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A series of bivalent thrombin inhibitors was synthesized, consisting of a d-phenylalanyl-prolyl-Nalpha(methyl)arginyl active site blocking segment, a fibrinogen recognition exosite inhibitor part, and a peptidic linker connecting these %%%fragments%%%. The methylation of the P1 amino acid led to a moderate decrease in affinity compared with the unmethylated analog. In addition, it prevented the thrombin catalyzed proteolysis, independent of the P1' amino acid used. This is a significant advantage compared to the original hirulogs, which strictly

require a proline as P1' amino acid to reduce the cleavage C-terminal to the arginyl residue. Several analogs were prepared by incorporation of different P1' amino acids found in natural thrombin substrates. The most potent inhibitor was I-11 (dCha-Pro-N(Me)Arg-Thr-(Gly)5-DYEPIPEEA-Cha-dGlu) with a Ki of 37 pM. I-11 is highly selective and no inhibition of the related serine proteases %%%trypsin%%%, factor Xa and plasmin was observed. The stability of I-11 in human plasma in vitro was strongly improved compared to hirulog-1. In addition, a significantly reduced plasma clearance of I-11 was observed after intravenous injection in rats. Results from molecular modeling suggest that a strong reorganization of the hydrogen bonds in the active site of thrombin may result in the proteolytic stability found in this inhibitor series.

2/7/4
DIALOG(R)File 5:Biosis Previews(R)

15715276 BIOSIS NO.: 200000433589
Incorporation of noncoded amino acids into the N-terminal domain 1-47 of %%%hirudin%%% yields a highly potent and selective thrombin inhibitor
AUTHOR: De Filippis Vincenzo; Russo Ilaria; Vindigni Alessandro; Di Cera Enrico; Salmaso Stefano; Fontana Angelo (Reprint)
AUTHOR ADDRESS: Department of Pharmaceutical Sciences, CRIBI Biotechnology Center, Viale G. Colombo 3, 35121, Padua, Italy**Italy
JOURNAL: Protein Science 8 (10): p2213-2217 Oct., 1999 1999
MEDIUM: print
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Hirudin%%% is an anticoagulant polypeptide isolated from a medicinal leech that inhibits thrombin with extraordinary potency (Kd = 0.2-1.0 pM) and selectivity. %%%Hirudin%%% is composed of a compact N-terminal region (residues 1-47, cross-linked by three disulfide bridges) that binds to the active site of thrombin, and a flexible C-terminal tail (residues 48-64) that interacts with the exosite I of the enzyme. To minimize the sequence of %%%hirudin%%% able to bind thrombin and also to improve its therapeutic profile, several N-terminal %%%fragments%%% have been prepared as potential anti-coagulants. However, the practical use of these %%%fragments%%% has been impaired by their relatively poor affinity for the enzyme, as given by the increased value of the dissociation constant (Kd) of the corresponding thrombin complexes (Kd = 30-400 nM). The aim of the present study is to obtain a derivative of the N-terminal domain 1-47 of %%%hirudin%%% displaying enhanced inhibitory potency for thrombin compared to the natural product. In this view, we have synthesized an analogue of %%%fragment%%% 1-47 of %%%hirudin%%% HM2 in which Val1 has been replaced by tert-butylglycine, Ser2 by Arg, and Tyr3 by beta-naphthylalanine, to give the BugArgNal analogue. The results of chemical and conformational characterization indicate that the synthetic peptide is able to fold efficiently with the correct disulfide topology (Cys6-Cys14, Cys16-Cys28, Cys22-Cys37), while retaining the conformational properties of the natural %%%fragment%%%. Thrombin inhibition data indicate that the effects of amino acid replacements are perfectly additive if compared to the singly substituted

analogues (De Filippis V, Quarzago D, Vindigni A, Di Cera E, Fontana A, 1998, Biochemistry 37:13507-13515), yielding a molecule that inhibits the fast or slow form of thrombin by 2,670- and 6,818-fold more effectively than the natural %%%fragment%%%, and that binds exclusively at the active site of the enzyme with an affinity (Kd,fast = 15.4 pM, Kd,slow = 220 pM) comparable to that of full-length %%%hirudin%%% (Kd,fast = 0.2 pM, Kd,slow = 5.5 pM). Moreover, BugArgNal displays absolute selectivity for thrombin over the other physiologically important serine proteases %%%trypsin%%%, plasmin, factor Xa, and tissue plasminogen activator, up to the highest concentration of inhibitor tested (10 muM).

2/7/5
DIALOG(R)File 5:Biosis Previews(R)

15369717 BIOSIS NO.: 200000088030
Anophelin: Kinetics and mechanism of thrombin inhibition
AUTHOR: Francischetti Ivo M B; Valenzuela Jesus G; Ribeiro Jose M C (Reprint)
AUTHOR ADDRESS: Medical Entomology Section, LPD, NIAID, 4 Center Dr., Bldg 4, Rm 126, Bethesda, MD, 20892-0425, USA**USA
JOURNAL: Biochemistry 38 (50): p16678-16685 Dec. 14, 1999 1999
MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Anophelin is a 6.5-kDa peptide isolated from the salivary gland of Anopheles albimanus that behaves as an alpha-thrombin inhibitor. In this paper, kinetic analyses and the study of mechanism of alpha-thrombin inhibition by anophelin were performed. Anophelin was determined to be a reversible, slow, tight-binding inhibitor of alpha-thrombin, displaying a competitive type of inhibition. The binding of anophelin to alpha-thrombin is stoichiometric with a dissociation constant (Ki) of 5.87 +- 1.46 pM, a calculated association rate constant (k1) of 2.11 +- 0.06 X 108 M-1 s-1, and a dissociation rate constant (k-1) of 4.05 +- 0.97 X 10-4 s-1. In the presence of 0.15 and 0.4 M NaCl, a 17.6- and 207-fold increase in the Ki of anophelin-alpha-thrombin complex was observed, respectively, indicating that ionic interactions are important in anophelin-alpha-thrombin complex formation. Incubation of alpha-thrombin with C-terminal %%%hirudin%%% %%%fragment%%% 54-65 that binds to alpha-thrombin anion binding exosite 1 (TABE1) attenuates alpha-thrombin inhibition by anophelin; anophelin also blocks TABE1-dependent %%%trypsin%%%-mediated proteolysis of alpha-thrombin. Using gamma-thrombin, an alpha-thrombin derivative where the anion binding exosite has been disrupted, anophelin behaves as a fast and classical competitive inhibitor of gamma-thrombin hydrolysis of small chromogenic substrate (Ki = 0.694 +- 0.063 nM). In addition, anophelin-gamma-thrombin complex formation is prevented by treatment of the enzyme with D-Phe-Pro-Arg-chloromethyl ketone (PPACK), a reagent that irreversibly blocks the catalytic site of thrombin. It is concluded that anophelin is a potent dual inhibitor of alpha-thrombin because it binds both to TABE1 and to the catalytic site, optimal binding being dependent on the availability of both domains. Finally, anophelin inhibits clot-bound alpha-thrombin with an IC50 of 45 nM and increases the lag

phase that precedes explosive in vitro alpha-thrombin generation after activation of intrinsic pathway of blood coagulation. Because of its unique primary sequence, anophelin may be used as a novel reagent to study the structure and function of alpha-thrombin.

2/7/6
DIALOG(R)File 5:Biosis Previews(R)

14914649 BIOSIS NO.: 199900174309
Allosteric modulation of BPTI interaction with human alpha- and zeta-thrombin
AUTHOR: De Cristofaro Raimondo (Reprint); Landolfi Raffaele
AUTHOR ADDRESS: Centro Ricerche Fisiopatologia dell'Emostasi, Istituto di Semeiotica Medica, Universita Cattolica, S. Cuore-Largo F. Vito 1, 00168, Roma, Italy**Italy
JOURNAL: European Journal of Biochemistry 260 (1): p97-102 Feb., 1999 1999
MEDIUM: print
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In this study, thrombin interaction with the basic pancreatic %%%trypsin%%% inhibitor (BPTI) was investigated in the presence of different allosteric modulators of thrombin, that is the C-terminal %%%hirudin%%% peptide 54-65 (Hir54-65), a recombinant thrombomodulin form (TMEGF4-6) and Na+. BPTI binding to alpha-thrombin is positively linked to Na+. Under low sodium concentration (5 mM Na+) the BPTI affinity for alpha-thrombin was roughly threefold lower than in the presence of 150 mM sodium (Ki = 320 muM vs. 100 muM). The %%%hirudin%%% %%%fragment%%%, which binds to the fibrinogen recognition site (FRS) of thrombin, induced a progressive and saturable decrease (3.6-fold) of alpha-thrombin affinity for BPTI, whereas the thrombomodulin peptide, which binds to a more extended region of FRS, caused a 5.5-fold increase of the enzyme affinity for the inhibitor. The opposite effect exerted by Hir54-65 and TMEGF4-6 was also observed for BPTI interaction with zeta-thrombin, in which the amidic bond between W148 and T149 is cleaved. However, in this case the effect by Hir54-65 and TMEGF4-6, although qualitatively similar to that observed with alpha-thrombin, had a smaller magnitude. Thrombin hydrolysis of Protein C was also differently affected by Hir54-65 and TMEGF4-6 peptides. While the latter enhanced the Protein C activation, the former caused a reduction of both alpha- and zeta-thrombin kcat/Km' for Protein C cleavage. These results showed that (a) Na+ facilitates BPTI interaction with thrombin; (b) Hir54-65 and TMEGF4-6, though sharing in part the same binding site at the thrombin FRS, can affect in opposite way thrombin's interaction with BPTI and Protein C; (c) such findings along with the results obtained with zeta-thrombin might be explained by admitting that the thermodynamic linkage between FRS and the critical W60-loop is also controlled by ligation and/or conformational state of the W148 insertion loop.

2/7/7
DIALOG(R)File 5:Biosis Previews(R)

14638684 BIOSIS NO.: 199800432931
Putative leech dopamine1-like receptor molecular characterization: Sequence homologies between dopamine and serotonin leech CNS receptors explain pharmacological cross-reactivities
AUTHOR: Salzet Beatrice; Stefano George B; Verger-Bocquet Martine; Salzet Michel (Reprint)
AUTHOR ADDRESS: Laboratoire d'Endocrinologie des Annelides, Groupe de Neuroimmunite des Hirudinees, SN3, Universite des Sciences et Technologies de Lille, F-58655 Villeneuve d'Ascq Cedex, France**France
JOURNAL: Molecular Brain Research 58 (1-2): p47-58 July 15, 1998 1998
MEDIUM: print
ISSN: 0169-328X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The biochemical characterization of a serotonin (5HT) receptor and the cloning of a dopamine (DA) receptor in the central nervous system (CNS) of the leech, Theromyzon tessulatum, is presented. Additionally, DA and 5HT binding sites were examined in the CNS by Scatchard analysis which showed a single, relatively high-affinity binding site with a Kd 1.1 nM and a Bmax 126 +- 18 fmol/mg protein for (3H)DA and a Kd 2.1 nM and a Bmax 225 fmol/mg protein for (3H)5HT. The first 88 amino acids of the 5HT receptor, isolated by a 5HT-affinity column followed by anion exchange chromatography and C3 reverse-phase HPLC exhibited a 43% sequence homology with Lymnaea stagnalis 5HT-receptor. The isolated DA receptor revealed a single protein of 45 kDa with an anti-DI-R in Western blot. The first 80 N-terminal amino acid residues and a %%%trypsin%%% digested %%%fragment%%% of 31 residues were obtained, and based on these sequencing data, a molecular biology strategy using reverse transcriptase-polymerase chain reaction, was developed. An amplified 1-kb segment was obtained. The complete deduced sequence of 416 amino acid residues exhibited about 30.6% sequence homology with the vertebrate D1 receptor family. Moreover, we further demonstrate that the leech 5HT and DA receptors also exhibit 30% sequence identity with each other, explaining their pharmacological cross-reactivity. Finally, anti-D1-R immunocytochemistry revealed positive structures in the peripheral and central nervous system, e.g., neurons, sensory fibers and immune cells. This is the first biochemical and molecular characterization of a DA receptor in leeches.

2/7/8
DIALOG(R)File 5:Biosis Previews(R)

13232543 BIOSIS NO.: 199698700376
Crystallographic determination of the structures of human alpha-thrombin complexed with BMS-186282 and BMS-189090
AUTHOR: Malley Mary F; Tabernero Lydia; Chang Chiehying Y; Ohringer Shari L ; Roberts Daniel G M; Das Jagabandhu; Sack John S
AUTHOR ADDRESS: Dep. Solid State Chemistry, Bristol-Myers Squibb Pharmaceutical Res. Inst., P.O. Box 4000, Princeton, NJ 08543-4000, USA** USA
JOURNAL: Protein Science 5 (2): p221-228 1996 1996
ISSN: 0961-8368

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The crystallographic structures of the ternary complexes of human alpha-thrombin with hirugen (a sulfated %%%hirudin%%% %%%fragment%%%) and the small-molecule active site thrombin inhibitors BMS-186282 and BMS-189090 have been determined at 2.6 and 2.8 ANG . In both cases, the inhibitors, which adopt very similar bound conformations, bind in an antiparallel beta-strand arrangement relative to the thrombin main chain in a manner like that reported for PPACK, D-Phe-Pro-Arg-CH-2Cl. They do, however, exhibit differences in the binding of the alkyl guanidine moiety in the specificity pocket. Numerous hydrophilic and hydrophobic interactions serve to stabilize the inhibitors in the binding pocket. Although PPACK forms covalent bonds to both serine and the histidine of the catalytic triad of thrombin, neither BMS-186282 nor BMS-189090 bind covalently and only BMS-186282 forms a hydrogen bond to the serine of the catalytic triad. Both inhibitors bind with high affinity (K-i = 79 nM and 3.6 nM, respectively) and are highly selective for thrombin over %%%trypsin%%% and other serine proteases.

2/7/9

12625523 BIOSIS NO.: 199598093356
Probing the structure of %%%hirudin%%% from %%%Hirudinaria%%% manillensis by limited proteolysis: Isolation, characterization and thrombin-inhibitory properties of N-terminal %%%fragments%%%
AUTHOR: Vindigni Alessandro; De Filippis Vincenzo; Zanotti Giuseppe; Visco Carlo; Orsini Gaetano; Fontana Angelo (Reprint)
AUTHOR ADDRESS: CRIBI Biotechnol. Centre, Via Trieste 75, I-35121 Padua, Italy**Italy
JOURNAL: European Journal of Biochemistry 226 (2): p323-333 1994 1994
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Hirudin%%% is the most potent and specific inhibitor of the blood-clotting enzyme thrombin so far known. Several %%%hirudin%%% variants were isolated mostly from Hirudo medicinalis and shown to be polypeptide chains of approximately 7 kDa with three internal disulfide bridges. In this study, limited proteolysis has been used to probe aspects of the structure and dynamics of a %%%hirudin%%% variant HM2 isolated from %%%Hirudinaria%%% manillensis. Proteolysis of the polypeptide chain of 64-amino-acid residues of %%%hirudin%%% HM2 by protease from Staphylococcus aureus V8, %%%trypsin%%%, thermolysin and subtilisin occurs at region 41-49 of the chain. The N-terminal %%%fragments%%% 1-41 and 1-47 were isolated to homogeneity and shown to maintain inhibitory action on thrombin, though much lower than the intact protein. The results were interpreted on the basis of a proposed three-dimensional structure of %%%hirudin%%% HM2 deduced by protein modelling the known structure of %%%hirudin%%% variant HV1 from Hirudo medicinalis (75% sequence similarity between HM2 and HV 1). Both proteolysis experiments and protein modelling provide evidence for the

existence in %%%hirudin%%% HM2 of a N-terminal well-structured domain (core) and a C-terminal flexible polypeptide segment. Determination of the accessible surface area of the three-dimensional model of %%%hirudin%%% HM2 showed that the sites of preferential cleavages are at the surface of the polypeptide molecule.

2/7/10
DIALOG(R)File 5:Biosis Previews(R)

12348956 BIOSIS NO.: 199497370241
Alpha-Thrombin and %%%trypsin%%% use different receptors to stimulate arachidonic acid metabolism
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JOURNAL: Prostaglandins 47 (6): p437-449 1994 1994
ISSN: 0090-6980
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Rat liver cells (the C-9 cell line) are stimulated to metabolize arachidonic acid by alpha-thrombin, its receptor polypeptide, gamma-thrombin, and %%%trypsin%%%. Prostaglandin (PG) I-2 synthesis stimulated by alpha-thrombin is inhibited by dansylarginine N-(3-ethyl-1,5-pentanediyl) amide (DAPA), by %%%hirudin%%%, by the synthetic tyrosine-sulfated dodecapeptide corresponding to residues 53-64 of %%%hirudin%%% (hirugen), by the Tyr(SO-3H)-63-%%%hirudin%%% %%%fragment%%% 54-65 and by rabbit lung thrombomodulin. Stimulation of arachidonic acid metabolism by the receptor octapeptide, SFLLRNPN, is not affected by DAPA or %%%hirudin%%%. gamma-Thrombin stimulates arachidonic acid metabolism but at 300 to 400-fold higher concentrations. %%%Trypsin%%% stimulates arachidonic acid metabolism. %%%Trypsin%%%'s proteolytic activity is required-its ability to stimulate is abolished if it is incubated with Na-p-tosyl-L-lysine chloromethyl ketone (TLCK) or bovine pancreatic %%%trypsin%%% inhibitor. Prior treatment of the rat liver cells with alpha-thrombin blocks subsequent stimulation by alpha-thrombin, but not by %%%trypsin%%%, whereas prior treatment with %%%trypsin%%% blocks subsequent stimulation by %%%trypsin%%%, but not the activity stimulated by alpha-thrombin. Prior treatment of the cells with the serine-proteases, chymotrypsin, pancreatic or neutrophil elastase and thrombocytin from Bothrups atrox venom, block alpha-thrombin's activation of PGI-2 production, but not the activity stimulated by %%%trypsin%%% These findings indicate that alpha-thrombin and %%%trypsin%%% stimulate PGI-2 production via different receptors.

2/7/11
DIALOG(R)File 5:Biosis Previews(R)

11911820 BIOSIS NO.: 199396076236
The complete amino acid sequence of a %%%Hirudin%%% variant from the Leech %%%Hirudinaria%%% manillensis
AUTHOR: Electricwala A (Reprint); Hartwell R; Scawen M D; Atkinson T
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JOURNAL: Journal of Protein Chemistry 12 (3): p365-370 1993
ISSN: 0277-8033
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Unlike the European leech Hirudo medicinalis, the Asian jawed leech %%%Hirudinaria%%% manillensis is specialized for feeding on mammalian blood. In the salivary glands of both these leeches, there is a potent inhibitor of thrombin, called %%%hirudin%%%, which acts as an anticoagulant. We have reported previously the isolation and purification of a variant of %%%hirudin%%%, called bufrudin, from the head portions of %%%Hirudinaria%%%. In the present study, the complete amino acid sequence of bufrudin was determined by automated Edman degradation of peptide %%%fragments%%% generated after cleavage of protein with %%%trypsin%%% or thermolysin. Comparison of the primary structure of bufrudin, with %%%hirudin%%% HV1, show about 70% sequence identity with deletion of two amino acids, but the key amino acids at the C-terminus, involved in the inhibition of thrombin, are conserved. However, similar sequence comparison of bufrudin with hirullin P18, a %%%hirudin%%% variant isolated from the same leech species but from whole leech, instead of heads, reveals even less sequence identity of about 60%. From the amino acid sequence, it is suggested that the conformation of the C-terminal portion of bufrudin may be significantly different from hirullin P18, but similar to %%%hirudin%%% HIV1, upon its interaction with thrombin. These results indicate that, as with Hirudo leech, various isoforms of %%%hirudin%%% also exist in %%%Hirudinaria%%% leech, with a significant change occurring in the structure of the molecular during the evolution of leeches.

2/7/12

10861019 BIOSIS NO.: 199192106790
REGULATION OF MESANGIAL CELL ADHESION AND SHAPE BY THROMBIN
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JOURNAL: American Journal of Physiology 261 (2 PART 2): pF336-F344 1991
ISSN: 0002-9513
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Adenosine 3',5'-cyclic monophosphate (cAMP) elevation in cultured rat mesangial cells causes urokinase-dependent adhesion loss, stress-fiber %%%fragmentation%%%, and shape change. Thrombin cleaves single-chain urokinase (scu-PA), causing its inactivation, but not two-chain u-PA [tcu-plasminogen activator (PA)] or tissue-type PA. We tested the ability of thrombin to inhibit the effects of cAMP elevation in mesangial cells and inactivate cell-associated scu-PA. In an assay of %%%trypsin%%%-sensitive adhesion, 65.9% of control cells and 5.5% of cells treated with isoproterenol + methylisobutylxanthine (IM) remained

adherent. In the presence of 0.01, 0.1, 1.0, and 10.0 unit/ml thrombin, 20.9, 46.6, 50.4 and 53.3%, respectively, of IM-treated cells remained attached. Thrombin also inhibited stress-fiber %%%fragmentation%%% and shape change. The effects of thrombin were blocked by %%%hirudin%%% or antithrombin III plus heparin. Direct zymography in gels containing gelatin and plasminogen revealed loss of a closely spaced pair of PA bands with thrombin treatment (1.0 unit/ml). %%%Hirudin%%% blocked the loss. .alpha.-Thrombin inactivated by diisopropyl fluorophosphate neither inhibited shape change nor caused loss of the PA bands; however, .gamma.-thrombin was nearly as active as native .alpha.-thrombin in both regards. Pretreatment of the cells with as little as 1.0 unit/ml thrombin for 1.0 min caused marked inhibition of shape change and near total loss of the slower migrating u-PA band (of the doublet). The faster migrating band was inhibited less. The results indicate that the slower migrating band represents scu-PA; the nature of the faster migrating band is less certain. Thrombin reversed the adhesion loss and shape change caused by 8-(4-chlorophenylthio)-cAMP and MIX. Thus physiological concentrations of thrombin rapidly inactivate mesangial cell scu-PA and inhibit and reverse cAMP-stimulated adhesion loss and shape change. Although inhibition of urokinase-dependent hydrolysis of extracellular matrix protein is apparently one mechanism by which thrombin regulates mesangial cell shape and adhesion, these observations are consistent with the possibility that cleavage of receptor-bound scu-PA by thrombin regulates these processses through signal transduction as well.

10318248 BIOSIS NO.: 199090102727
DESIGN AND CHARACTERIZATION OF HIRULOGS A NOVEL CLASS OF BIVALENT PEPTIDE INHIBITORS OF THROMBIN
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JOURNAL: Biochemistry 29 (30): p7095-7101 1990
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A novel class of synthetic peptides has been designed that inhibit the thrombin catalytic site and exhibit specificity for the anion-binding exosite (ABE) of .alpha.-thrombin. These peptides, called "hirulogs", consist of (i) an active-site specificity sequence with a restricted Arg-Por scissile bond, (ii) a polymer linker of glycyl residues from 6 to 18 .ANG. in length, and (iii) an ABE recognition sequence such as that in the %%%hirudin%%% C-terminus. Hirulog-1 [(D-Phe)-Pro-Arg-Por-(Gly)4-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Tyr-Leu] inhibits the thrombin-catalyzed hydrolysis of a tripeptide p-nitroanilide substrate with Ki = 2.3 nM. In contrast, the synthetic C-terminal %%%hirudin%%% peptide S-Hir53-64, which binds to the thrombin ABE, blocked the fribinogen clotting activity of the enzyme with Ki = 144 nM but failed to inhibit the hydrolysis of p-nitroanilide substrates at concentrations as high as 1 mM. In addition, the pentapeptide

(D-Phe)-Pro-Arg-Pro-Gly, which comprises the catalytic-site inhibitor moiety of hirulog-1, was determined to have a Ki for thrombin inhibition >2 .mu.M. Hirulog-1, but not S-Hir53-64, was found to inhibit the incorporation of [14C]diisopropyl fluorophosphate in thrombin. Hirulog-1 appears specific for thrombin as it lacks inhibitory activities toward human factor Xa, human plasmin, and bovine %%%trypsin%%% at inhibitor:enzyme concentrations 3 orders of magnitude higher than those required to inhibit thrombin. The optimal inhibitory activity of hirulog-1 depends upon all three components of its structure. Hirulog-1 inhibited human .gamma.-thrombin and bovine thrombin with Ki values increased 500- and 20-fold, respectively, compared to Ki for human .alpha.-thrombin. Also, hirulog-1 inhibition of .alpha.-thrombin was reversed in the presence of saturating concentrations of S-Hir53-64. Studies on the optimal length of the oligoglycyl spacer, which forms a molecular "bridge" linking active-site and ABE recognition sequences, showed that at least three to four glycines were necessary for optimal inhibitory activity. Comparison of anticoagulant activities of hirulog-1, %%%hirudin%%%, and S-Hir53-64 showed that the synthetic hirulog-1 is 2-fold more potent than %%%hirudin%%% and 100-fold more active than S-Hir53-64 in increasing the activated partial thromboplastin time of normal human plasma. Thus, fashioned from studies on %%%hirudin%%% and its %%%fragments%%%, synthetic peptides that bind to both the ABE and catalytic site of thrombin are potent reversible inhibitors of thrombin activities.

10185562 BIOSIS NO.: 199089103453
USE OF %%%FRAGMENTS%%% OF %%%HIRUDIN%%% TO INVESTIGATE THROMBIN-%%%HIRUDIN%%% INTERACTION
AUTHOR: DENNIS S (Reprint); WALLACE A; HOFSTEENGE J; STONE S R
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JOURNAL: European Journal of Biochemistry 188 (1): p61-66 1990
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Site-directed mutagenesis was used to create %%%hirudin%%% in which Asn52 was replaced by methionine. Cyanogen bromide cleavage at this unique methionine resulted in two %%%fragments%%%. These %%%fragments%%% have been used to study the kinetic mechanism of the inhibition of thrombin by %%%hirudin%%% and to identify areas of the two molecules which interact with each other. The binding of the C-terminal %%%fragment%%% (residues 53-65) to thrombin resulted in a decrease in the Michaelis constant for the substrate D-phenylalanylpipecolylarginyl-p-nitroanilide (DPhe-Pip-Arg-NH-Ph). The N-terminal %%%fragment%%% (residues 1-52) was a competitive inhibitor of thrombin. There was a small amount of cooperativity in the binding of the two %%%fragments%%%. Whereas %%%hirudin%%% and its C-terminal %%%fragment%%% protected .alpha.-thrombin against cleavage by %%%trypsin%%%, the N-terminal %%%fragment%%% did not. %%%Hirudin%%% and the N-terminal %%%fragment%%% completely prevented the cleavage of

.alpha.-thrombin by pancreatic elastase while the C-terminal %%%fragment%%% afforded a lesser degree of protection. The results of these experiments with %%%trypsin%%% and elastase are discussed in terms of interaction areas on thrombin and %%%hirudin%%%.

2/7/15
DIALOG(R)File 5:Biosis Previews(R)

10148770 BIOSIS NO.: 199089066661
DISTINCT BINDING SITES OF ALA-48 %%%HIRUDIN%%%-1-47 AND ALA-48 %%%HIRUDIN%%%-48-65 ON ALPHA THROMBIN
AUTHOR: DODT J (Reprint); KOEHLER S; SCHMITZ T; WILHELM B
AUTHOR ADDRESS: INST FUER BIOCHEMIE, TECHNISCHE HOCHSCHULE DARMSTADT, PETERSENSTRASSE 22, D-6100 DARMSTADT, WEST GERMANY**WEST GERMANY
JOURNAL: Journal of Biological Chemistry 265 (2): p713-718 1990
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The interaction of .alpha.-thrombin with Ala48-%%%hirudin%%%, Ala48-%%%hirudin1%%%-47, and Ala48-%%%hirudin48%%%-65 was analyzed. Mutations at Pro48 were found to cause only slight changes in the kon (human: 3.1 .+-. 0.3 .times. 108 M-1 s-1; bovine: 1.03 .+-. 0.3 .times. 108 M-1 s-1) and koff (human: 0.4 .+-. 0.2 .times. 10-3 s-1; bovine: 2.9 .+-. 0.4 .times. 10-3 s-1) rate constants for the formation of the thrombin-%%%hirudin%%% complex. The amino-terminal %%%fragment%%% Ala48-%%%hirudin1%%%-47 containing the three disulfide bridges and the carboxyl-terminal %%%fragment%%% Ala48-%%%hirudin48%%%-65 were derived from the Ala48 mutant by proteolysis with endoproteinase Lys-C. These %%%fragments%%% inhibit bovine .alpha.-thrombin clotting activity with IC50 values of 0.6 and 4.9 .mu.M, respectively (2.4 nM for r-%%%hirudin%%%). By mapping the interaction of Ala48-%%%hirudin%%%-derived %%%fragments%%% with bovine .alpha.-thrombin by limited proteolysis with %%%trypsin%%% and pancreatic elastase distinct binding sites for each %%%fragment%%% were determined. The carboxyl-terminal %%%fragment%%% was found to bind to the proposed anion-binding exosite in the region B62-74, whereas the amino-terminal %%%fragment%%% binds to a region around the elastase cleavage site at residues 150-151 of the .alpha.-thrombin B-chain.

2/7/16
DIALOG(R)File 5:Biosis Previews(R)

09552884 BIOSIS NO.: 198987000775
DISSOCIATION OF FIBRINOGEN AND FIBRONECTIN BINDING FROM TRANSGLUTAMINASE-MEDIATED CROSS-LINKING AT THE HEPATOCYTE SURFACE
AUTHOR: BARSIGIAN C (Reprint); FELLIN F M; JAIN A; MARTINEZ J
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JOURNAL: Journal of Biological Chemistry 263 (28): p14015-14022 1988
ISSN: 0021-9258
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The interaction of fibrinogen and fibronectin with hepatocytes has been dissociated into distinct binding and cross-linking steps. Binding and cross-linking of 125I-labeled ligands were both decreased by transglutaminase inhibitors, but not by heparin or %%%hirudin%%%. Transglutaminase activity was manifest by Ca2+-dependent incorporation of [14C]putrescine into cells. Preferential cross-linking of fibrinogen A.alpha. over .gamma. chains, and lack of inhibition by heparin or %%%hirudin%%% indicates the involvement of tissue transglutaminase, and not Factor XIIIa. Hepatic transglutaminase activity, as well as binding and cross-linking of fibrinogen and fibronectin, were maximally supported by Ca2+, partially supported by Mn2+ and Sr2+, and markedly decreased by Mg2+ and Ba2+. In contrast, Co2+ supported binding but not cross-linking or transglutaminase activity, indicating that binding and cross-linking are dissociable events. This conclusion was corroborated by the finding that fibrinogen %%%fragments%%% D95 and D78 both inhibited Ca2+-dependent fibrinogen binding without being cross-linked themselves. Ligand binding in the presence of either cation was localized to the cell surface as evidenced by its %%%trypsin%%% sensitivity. Thus, fibrinogen and fibronectin binding to hepatocytes is independent of transglutaminase activity, whereas cross-linking of these adhesive macromolecules requires an enzymatically active cellular transglutaminase. In addition, fibrinogen binding appears to be mediated by molecular determinants present in %%%fragment%%% D78.

2/7/17

09096850 BIOSIS NO.: 198885065741
CHARACTERIZATION AND PURIFICATION OF A PROTEASE IN SERUM THAT CLEAVES PROATRIAL NATRIURETIC FACTOR PROANF TO ITS CIRCULATING FORMS
AUTHOR: ZISFEIN J B (Reprint); GRAHAM R M; DRESKIN S V; WILDEY G M; FISCHMAN A J; HOMCY C J
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JOURNAL: Biochemistry 26 (26): p8690-8697 1987
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Atrial natriuretic factor (ANF) is synthesized and stored in atrial cardiocytes as a 17-kilodalton (kDa), 126 amino acid polypeptide, proANF, but circulates as smaller, 24 and 28 amino acid peptide %%%fragments%%% of the carboxy terminus of proANF. It has previously been shown that proANF is secreted intact from cultured atrial cardiocytes and can be cleaved by a serum protease to smaller, 3-kDa peptides believed to be the circulating forms. This report describes the purification and characterization of this proANF-cleaving protease from rat serum. The cleavages both of 35S-labeled proANF derived from rat atrial cell cultures, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/autoradiography, and of a synthetic p-nitroanilide-containing substrate were used as assays for the detection

of enzyme activity. ProANF-cleaving activity was found in rat serum, with no such activity detectable in rat plasma. Cleavage in serum was not dependent on the presence of platelets or other cellular elements. Complete inhibition of proANF cleavage was obtained with the protease inhibitors benzamidine, leupeptin, phenylmethanesulfonyl fluoride, and diisopropyl fluorophosphate (DFP) but not with aprotinin, soybean %%%trypsin%%% inhibitor, pepstatin, or %%%hirudin%%%. Unlike the vitamin K dependent plasma proteins, the proANF-cleaving protease did not adsorb to barium sulfate. With the sequential application of ion-exchange, hydroxylapatite, lectin affinity, and gel filtration chromatography, a 5000.sbd.6000-fold purification of the enzyme from rat serum was achieved. Fractionation of either whole serum or the purified enzyme by gel filtration chromatography revealed a single peak of activity corresponding to a protein with a Stokes radius of 45 .ANG.. The pI of the enzyme was found to be approximately 5.6. Incubation of the purified enzyme with [3H]DFP followed by SDS-PAGE and autoradiography revealed a specifically labeled 38-kDa peptide, the substrate binding subunit. Analysis by high-performance liquid chromatography of the 3-kDa products resulting from the cleavage of 35S-labeled proANF by the purified enzyme revealed, as previously described with whole serum, two radiolabeled peptides which coeluted with the 28 and 24 amino acid C-terminal preptides. Moreover, a time-dependent increase in the abundance of the latter peptide was found. These observations imply a precursor-product relationship, with the initial cleavage of proANF to the 28 amino acid peptide, which is then cleaved to the 24 amino acid peptide. These studies indicate that the majority of proANF cleavage activity found in rat serum is represented by that of a distinct serine protease whose properties are different from a variety of well-characterized proteases, such as kallikrein, plasmin, and the vitamin K dependent plasma proteins. The role of this protease in the in vivo processing of proANF remains to be defined.

08184592 BIOSIS NO.: 198682030979
PROTEOLYTIC CLEAVAGE OF HUMAN VON WILLEBRAND FACTOR INDUCED BY ENZYMES RELEASED FROM POLYMORPHONUCLEAR CELLS
AUTHOR: THOMPSON E A (Reprint); HOWARD M A
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JOURNAL: Blood 67 (5): p1281-1285 1986
ISSN: 0006-4971
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In vivo %%%fragmentation%%% of the von Willebrand factor antigen (vWF:Ag) molecule has been demonstrated on radiocrossed immunoelectrophoresis (CIE) in the plasma from patients with disseminated intravascular coagulation, in factor VIII concentrates, and in normal serum. Experiments reported here show that polymorphonuclear (PMN) cells contain a non-calcium-dependent protease(s) that when released and incubated with vWF:Ag results in an additional vWF:Ag peak on radio-CIE. Production of %%%fragments%%% of vWF:Ag by incubation with PMN cells

occurred in a time-dependent manner. The protease(s) responsible was inhibited by diisopropyl fluorophosphate, soybean %%%trypsin%%% inhibitor, and aprotinin, but not by benzamidine, azide, epicron, or %%%hirudin%%%. Citrate, EDTA, and leupeptin also had no effect on the PMN cell enzyme's activity, indicating that the enzyme(s) is not calcium dependent. The PMN cell enzyme responsible for vWF:Ag %%%fragmentation%%% is located intracellularly and released by freeze-thaw lysis or cell activation by calcium or the calcium ionophore A23187.

2/7/19
DIALOG(R)File 5:Biosis Previews(R)

07673355 BIOSIS NO.: 198579092254
CLEAVAGE OF HUMAN VON WILLEBRAND FACTOR BY PLATELET CALCIUM-ACTIVATED PROTEASE
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JOURNAL: Blood 65 (2): p352-356 1985
ISSN: 0006-4971
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In human platelet lysates prepared by addition of nonionic detergent (Triton X-100) or by sonication, the multimer composition and electrophoretic mobility of platelet von Willebrand factor (vWF) were consistently modified under conditions that would favor activation of the endogenous Ca-activated, sulfhydryl-dependent neutral protease (CAP). By sodium dodecylsulfate-agarose gel electrophoresis, native platelet vWF contained some multimers that were larger than those characteristic of plasma vWF. Modified platelet vWF contained a multimer population equivalent to or smaller than that of plasma vWF plus an additional fast-migrating band. In crossed immunoelectrophoresis (CIE), modified platelet vWF was characterized by a more anodic distribution and the appearance of a distinct, cross-reactive, anodic component previously designated VIIIR:Ag %%%fragment%%%. In the presence of Ca, radiolabeled purified plasma vWF was also degraded by the protease in question, with a decrease in the apparent MW of the reduced monomer from 230,000-205,000. The VIIR:Ag %%%fragment%%% isolated from the same degraded plasma vWF by preparative CIE was shown to be composed of an identical MW 205,000 subunit. Because cleavage of plasma or platelet vWF was inhibited by prior addition of leupeptin, EDTA, ethylene glycol bis (.beta.-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), or N-ethylmaleimide (agents known to inhibit platelet CAP) but was unaffected by numerous other protease inhibitors, including soybean %%%trypsin%%% inhibitor, benzamidine, %%%hirudin%%%, phenylmethylsulfonyl fluoride, aprotonin or .epsilon.-aminocaproic acid (none of which inhibits platelet CAP), proteolysis of vWF observed in this study apparently is a direct effect of CAP and is not mediated by way of secondary proteases.

2/7/20
DIALOG(R)File 5:Biosis Previews(R)

07597689 BIOSIS NO.: 198579016588
SUBTILISIN INHIBITOR FROM SEEDS OF BROAD BEAN VICIA-FABA CULTIVAR KLEINE-THUERINGER PURIFICATION AMINO-ACID SEQUENCE AND SPECIFICITY OF INHIBITION
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AUTHOR ADDRESS: CARLSBERG LAB, DEP CHEM, GAMLE CARLSBERG VEJ 10, DK-2500 COPENHAGEN VALBY**DENMARK
JOURNAL: Carlsberg Research Communications 49 (4): p493-502 1984
ISSN: 0105-1938
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A potent inhibitor of microbial serine proteases, including subtilisin, was purified 1100-fold from seeds of broad bean. Chymotrypsin and %%%trypsin%%% were not inhibited, but a weak temporary inhibition of pancreas elastase was observed. The preparation of pure inhibitor contained 1 major molecular form with a blocked N-terminal (MW .apprx. 10,000, pI .apprx. 4.8) and a minor form (< 10%) with slightly lower MW and pI .apprx. 5.0. About 80% of the amino acid sequence of the major form was determined by automatic Edman degradation of a cyanogen bromide %%%fragment%%% and tryptic peptides. The inhibitor is homologous with barley, potato and leech inhibitors of the potato inhibitor I family (36-56% of the amino acid residues in identical positions). Cleavage studies suggest one enzyme inhibitory site at an Ala.sbd.Asp bond.

2/7/21
DIALOG(R)File 5:Biosis Previews(R)

05914023 BIOSIS NO.: 198069028010
THROMBOCYTIN A SERINE PROTEASE FROM BOTHROPS-ATROX VENOM 2. INTERACTION WITH PLATELETS AND PLASMA CLOTTING FACTORS
AUTHOR: NIEWIAROWSKI S (Reprint); KIRBY E P; BRUDZYNSKI T M; STOCKER K
AUTHOR ADDRESS: DEP MED, SPEC CENT THROMB RES, TEMPLE UNIV HEALTH SCI CENT, PHILADELPHIA, PA 19140, USA**USA
JOURNAL: Biochemistry 18 (16): p3570-3577 1979
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Thrombocytin, a serine protease from B. atrox venom, caused platelet aggregation and release of platelet constituents at a concentration of 10-7 M and clot retraction at a concentration of 2 .times. 10-9 M. Thrombocytin was slightly more active when tested on platelets in plasma than on washed platelets suspended in Tyrode-albumin solution. Thrombin was 5 times more active than thrombocytin when tested on platelets in plasma and 50 times more active when tested on washed platelets. Release patterns induced by thrombocytin and thrombin were similar. Prostaglandin E1 (10-5 M) produced complete inhibition of platelet release induced by thrombocytin and thrombin. Indomethacin (10-4 M) was without any effect. Antithrombin III, in the presence of heparin, inhibited the action of thrombocytin on platelets and on a synthetic peptide substrate (Tos-Gly-Pro-Arg-pNA .cntdot. HCl). Formation of an

antithrombin III-thrombocytin complex was demonstrated on NaDodSO4-polyacrylamide gel electrophoresis. %%%Hirudin%%% and .alpha.1-antitrypsin did not inactivate thrombocytin. Thrombocytin had a low fibrinogen-clotting activity (less than 0.06% that of thrombin). Thrombocytin also caused progressive degradation of the .alpha. chain of human fibrinogen, and it cleaved prothrombin, releasing products similar to intermediate 1 and %%%fragment%%% 1 produced by thrombin. Thrombocytin activated factor XIII by limited proteolysis and increased the procoagulant activity of factor VIII in a manner analogous to that of thrombin.

04939296 BIOSIS NO.: 197662035435
CHARACTERISTICS OF THE ASSOCIATION BETWEEN PROTHROMBIN %%%FRAGMENT%%% 2 AND ALPHA THROMBIN
AUTHOR: MYRMEL K H; LUNDBLAD R L; MANN K G
JOURNAL: Biochemistry 15 (8): p1767-1773 1976
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Unspecified

ABSTRACT: The esterolytic activity of bovine .alpha.-thrombin on the synthetic substrate N-.alpha.-p-tosyl-L-arginine methyl ester (TosArgOMe) was stimulated when the prothrombin activation %%%fragment%%%, prothrombin %%%fragment%%% 2, was added as previously reported. A similar stimulation of .beta.-thrombin was observed upon addition of prothrombin %%%fragment%%% 2. The dissociation constant was 7.7 .times. 10-10 M and there was 1 molecule of prothrombin %%%fragment%%% 2 bound per molecule of .alpha.-thrombin. Prethrombin-2 competed for prothrombin %%%fragment%%% 2, so the enhancement of the esterolytic activity of .alpha.-thrombin by prothrombin %%%fragment%%% 2 was used as a probe to determine the dissociation constant for the binding of prothrombin %%%fragment%%% 2 to prethrombin 2. The dissociation constant for this association was 1.3 .times. 10-10 M. The kinetic parameters for the reaction of .alpha.-thrombin on TosArgOMe were determined in the absence and presence of prothrombin %%%fragment%%% 2. In the absence of prothrombin %%%fragment%%% 2, Km(app) = 1.92 .times. 10-4 M, and k3(app) = 35.8 mol of TosArgOMe/mol of .alpha.-thrombin s-1; in the presence of prothrombin %%%fragment%%% 2, Km(app) = 1.76 .times. 10-4 M, and k3(app) = 60.5 mol of TosArgOMe/mol of .alpha.-thrombin s-1. The stimulatory effect of bovine prothrombin %%%fragment%%% 2 on bovine .alpha.-thrombin was reflected in k3(app) and not in Km(app). In contrast to the stimulatory effect of prothrombin %%%fragment%%% 2 on the thrombin-catalyzed hydrolysis of TosArgOMe, it inhibited the activity of .alpha.-thrombin toward N-.alpha.-benzoyl-L-arginine ethyl ester and N-.alpha.-benzoyl-L-arginine p-nitroanilide. The inhibition of activity toward these substrates by prothrombin %%%fragment%%% 2 was also reflected in k3(app). Activity toward the nonspecific substrate p-nitrophenyl butyrate was completely inhibited by the addition of prothrombin %%%fragment%%% 2. Prothrombin %%%fragment%%% 2 had no effect on the inhibition of .alpha.-thrombin activity by the active-site serine inhibitors diisopropyl phosphofluoridate, phenylmethanesulfonyl fluoride,

or p-nitrophenyl guanidinobenzoate. Inhibition by the active-site-histidine-modifying inhibitor, N-.alpha.-p-tosyl-L-arginine chloromethyl ketone, was enhanced by the addition of prothrombin %%%fragment%%%. Soybean %%%trypsin%%% inhibitor reduced the stimulation by prothrombin %%%fragment%%% 2, but only at high molar ratios. Prothrombin %%%fragment%%% 2 had no effect on the clotting activity of .alpha.-thrombin, nor inhibition of this activity by heparin, %%%hirudin%%%, or diisopropyl phosphofluoridate. Bovine prothrombin %%%fragment%%% 2 enhanced the esterolytic activity of both human and bovine .alpha.-thrombin, but human prothrombin %%%fragment%%% 2 did not enhance the esterolytic activity of either human or bovine .alpha.-thrombin.

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